

nature*December 11, 1975*

A real forum for scientists

THE election of a new President of the Royal Society for a five-year term is an appropriate occasion on which to ask what part Britain's academy of science should play in the scientific life of the nation. The society has been evolving, not only under pressure from outside—it has never lacked vociferous critics—but also under the influence of successive presidents, and it is certainly not beyond its capacity to change even more. But in what direction? Here is one small suggestion.

Rank-and-file Fellows frequently complain that its operations are centred too much on London and are under the control of its Council to an excessive extent. (Those who dispute this latter statement have only to look at the process by which the President is 'elected'.) Fortunately, however, British science as a whole has never seriously suffered from the general tendency to accumulate power and influence in the nation's capital. The strength of the universities has guaranteed that, and however much the bureaucracy might accrete in one

place, the intellectual power has been widely scattered.

But universities are not what they were even a few years ago, and the importance of the central bureaucracy is undoubtedly growing. What more imaginative gesture could the Royal Society make in these circumstances than to move some of its activities outside London? These could well include parts of its committee work, but much more generally valuable would be the organisation in the provinces of regular discussion meetings—which need not be major gatherings such as the ones the society has successfully encouraged in the past few years.

There is a need for some forum in which scientists could discuss, at a local level and away from the public gaze, the problems of practising science. Not all of these problems are narrow technical ones; many concern funding, policy, science and society, education and so on. Were the Royal Society to organise regular meetings out of London to discuss these matters, it would be doing a great service in bringing scientists closer together.

Fearless, blunt message to our readers

OUR ultimate objective is to continuously assess and ultimately hopefully optimise the degree of clarification inherent in the information-conveying procedure currently selected by scientific practitioners.

At this present time we envision subjecting all written communication to a rigorous and ongoing scrutiny process. The management sees as its mission a systematic evaluation of, or at least an evaluative role in, the determinative processes by which overly extended documentation is submitted to a continuous questioning of its appropriate modalities. Where these do not parallel, or even sub-parallel, currently acceptable norms, the documentation will be the subject of an operationally proven interactive process in which the submitter and a qualified representative of this communication channel group will have as their function the production of a sanitised, re-evaluated word-stream.

To this end, attendees at such encounters should familiarise themselves with the logistics and algorithms currently utilised and should show an awareness of the

editorial systems that as of now we have identified as of most central relevance and impact.

The problem-oriented environment to which we address ourselves does not permit us to essentially guarantee continuous relative stabilisation with respect to our operating procedure paradigms. Future changes may be effectuated when anomalistic situations arise and have undergone assessment; the re-evaluation phase will characteristically be followed subsequently by an implementation of revised and updated procedures and/or systems. Our ultimate objective must always be the optimisation of the two-way information flow, if necessary by innovative support techniques.

We underscore our conviction that the utilisation of word-processing equipment for manuscript generation has to be viewed as a major, not to say a prime, necessity.

At this time the personnel associated with this information-dissemination mode convey to its recipients appropriate greetings for the festive period upcoming momentarily.



Naming the Loch Ness monster

Recent publicity concerning new claims for the existence of the Loch Ness monster has focused on the evidence offered by Sir Peter Scott and Robert Rines. Here, in an article planned to coincide with the now-cancelled symposium in Edinburgh at which the whole issue was due to be discussed, they point out that recent British legislation makes provision for protection to be given to endangered species; to be granted protection, however, an animal should first be given a proper scientific name.

Better, they argue, to be safe than sorry; a name for a species whose existence is still a matter of controversy among many scientists is preferable to none if its protection is to be assured. The name suggested is *Nessiteras rhombopteryx*.

SCHEDULE 1 of the Conservation of Wild Creatures and Wild Plants Act, 1975, passed recently by the UK Parliament, provides the best way of giving full protection to any animal whose survival is threatened. To be included, an animal should be given a common name and a scientific name. For the Nessie or Loch Ness monster, this would require a formal description, even though the creature's relationship with known species, and even the taxonomic class to which it belongs, remain in doubt.

On August 8, 1972, a team from the Academy of Applied Science, Boston, Massachusetts, working in conjunction with the Loch Ness Investigation Bureau of London, obtained what seems to be the most precise evidence on which to base such a description.

Two consecutive underwater photographs (Fig. 1) were taken by a stationary time-lapse camera with strobe flash, operating automatically at a depth of 45 feet in Loch Ness, along with a simultaneous sonar trace (Fig. 2). The photographs have been computer enhanced at the Jet Propulsion Laboratory in Pasadena, California, a technique which can 'improve' the image by comparing adjacent grains electronically so as to remove haziness, but cannot alter shapes or otherwise falsify the record.

A black triangle in one corner of the photograph is caused by the edge of the strobe flash apparatus, and should be disregarded. The pictures show a flattened, diamond-shaped fin, flipper or paddle, in which the limb structure is not quite central. Calculations from optical data corroborated by simultaneous sonar recordings suggest that the paddle is about 2 m long. Given its function, the 'main spar' of the paddle is likely to be nearer the leading, rather than the trailing edge, suggesting that it is a right-sided paddle.

A neck would be likely anterior to a forelimb, and a wider body posterior to it; since the opposite appears to be the case the photographs are assumed to show a right hind limb. The strobe

light illuminates an area of the animal's back and belly with a rough skin-texture. In the upper photograph there is what may be some suggestion of ribs.

Although these two photographs of the hind flipper are the main basis of the description, and the flipper-length is thought to be some 2 m, it is possible, using the evidence from other photographs and from sightings, to indicate some further features and dimensions of the animal. A total body length of 15–20 m seems possible including a neck of 3–4 m with a rather small head which may have some horn-like protuberances. Moving-target-discriminating sonar displays have provided body length measurements of the order of 15 m, and the underwater automatic strobe photography has provided support for the reports of a long neck.

Frequent descriptions liken the back to 'an up-turned boat', and both still photographs and films show this configuration. Further underwater photographs taken in June 1975 may show other aspects of the same species, including a view of the head, neck and body (Fig. 3). The Loch Ness monster may possibly resemble the impression shown in Figs 4 and 5.

It is proposed that the large animal species living in Loch Ness be called *Nessiteras rhombopteryx*, Scott and Rines (nov. genus and species; the only species is automatically the type species) with the common names: the Nessie or Loch Ness monster. The generic name *Nessiteras*, a neuter noun, is a composite word combining the name of the Loch with the Greek word *teras*, genitive *teratos*, which was used from Homer onwards to mean a marvel or wonder, and in a concrete sense for a range of monsters which aroused awe, amazement and often fear. The specific name *rhombopteryx* is a combination of the Greek *rhombos*, a diamond or lozenge shape, and the Greek *pteryx* meaning a fin or wing. Thus the species is the Ness monster with diamond fin.

In trying to determine which class

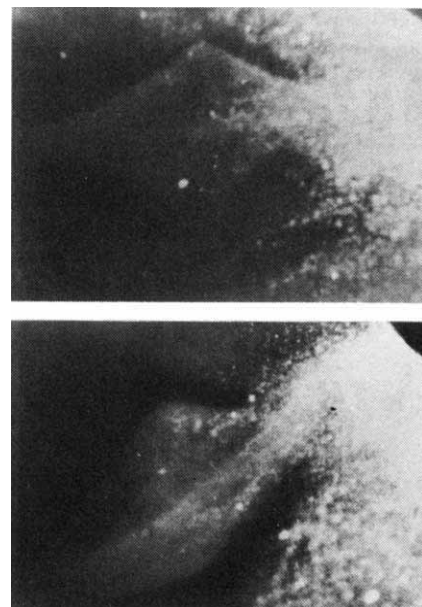


Fig. 1 Photographs taken by strobe flash at a depth of 45 feet in Loch Ness at 0150 h on August 8, 1972, showing the right hind flipper, calculated as about 2 m long, of *Nessiteras rhombopteryx*. The lower picture was taken about 1 min after the upper. The camera was stationary and aimed horizontally. The photographs were taken with equipment devised by Professor Harold Edgerton of the Massachusetts Institute of Technology and have been computer enhanced at the Jet Propulsion Laboratory, Pasadena, California. (Copyright, Academy of Applied Science, Boston, Massachusetts.)



Fig. 2 Sonar trace (Raytheon DE 725 C) of the period when the photographs in Fig. 1 were taken. The sonar set was aimed horizontally, and the strong echoes are at a range of about 40 metres. Sonar frequency 200 kHz. Time marks on the right of the picture are at 5 min intervals. The arrows mark the period during which the photographs were taken. The indications are that two large animals were present.

they belong to, it is concluded that the paddle in Fig. 1 must belong to a vertebrate animal. It has been established that no living cetacean has such a flipper, and the inclination is to view it as reptilian. Further designation must be pure speculation.

It is clearly unsatisfactory, from a zoological point of view, to base a name on photographs rather than on the remains of an animal, or at least some part of it. This means that, for the time being, there is no 'holotype' or 'type-specimen'. But description from an illustration is permitted by the International Code of Zoological Nomenclature, and the procedure seems justified by the urgency of comprehensive conservation measures.

The name proposed does not link the species to any animal or group of animals known to science. It applies to the animal first recorded in Loch Ness at the time of St Columba's visit in 565 AD and perhaps also to similar animals reported in other freshwater lakes in Scotland and elsewhere. *Nessiteras rhombopteryx* probably also has affinities with some fossil marine forms and perhaps also with living 'sea serpents' which have been named in the past. In 1817 Rafinesque named the Massachusetts Bay sea serpent *Megophias monstrosus*. In 1892 Oudemans changed the name to *Megophias megophias* and in 1958 Heuvelmans proposed *Megalotaria longicollis* for the animal sighted in the surrounding seas. We see no reason to suppose that this animal is conspecific or even congeneric with the animals in Loch Ness, and we do not therefore think the names used by Rafinesque, Oudemans and Heuvelmans apply to the owner of the hind flipper in the photographs.

The 'population density' of the Nessies is no doubt dictated by the size of the loch and the abundance of food. Loch Ness is 24 miles long, up to 1.5 miles wide, and 700 feet deep over much of its length, with a deepest point so far discovered of 975 feet. The surface is 50 feet above sea level. Salmon, sea trout and elvers running up the River Ness into the loch can thereafter swim up a number of rivers which run into it, and salmon, sea trout and well grown eels must descend these rivers into the loch on their way back to the sea. There are also resident populations of brown trout, char and sticklebacks. The shallow waters are well grown with freshwater weeds, and organic detritus must also be considered as a possible food source.

It has been calculated that the biomass of the loch could support a population of large animals. Between the melting ice and the present time the loch was probably, rather briefly, an arm of the sea. A population may

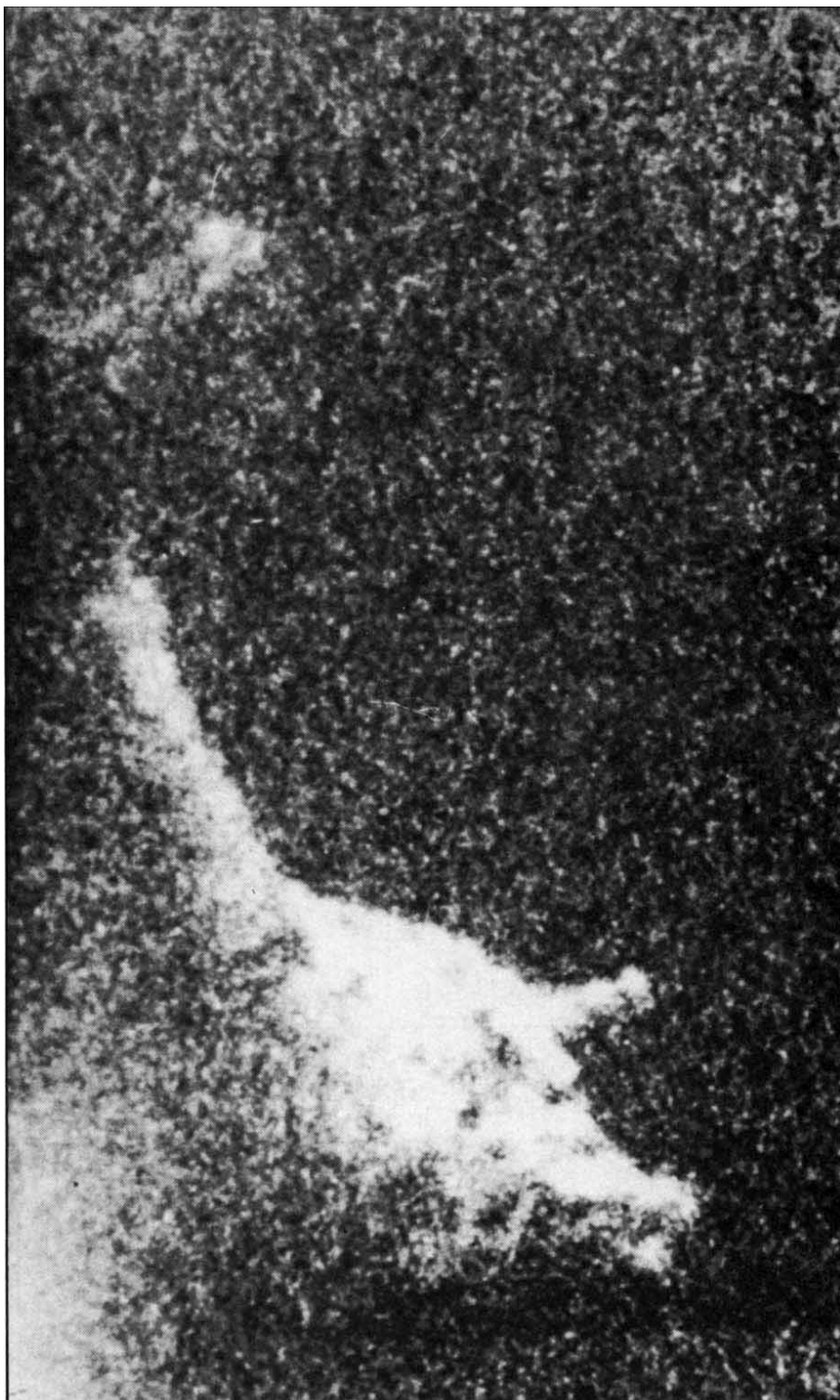


Fig. 3 Photograph taken by strobe flash at a depth of 35 feet in 80 feet or more of water in Loch Ness at 0430 h on June 20, 1975, showing the head and neck (7-12 feet in length) together with part of the body, with appendages, of *Nessiteras rhombopteryx*. The main body structure is 20-25 feet from the camera which is pointing about 30° above horizontal. Adjacent frames, taken about 1 min before and 1 min after, show nothing.

(Copyright, Academy of Applied Science, Boston, Massachusetts.)

therefore have become landlocked some 12,000 years ago. The possibility that juvenile animals may have ascended the 5 miles of the River Ness more recently seems less likely. Viable populations of vertebrate animals have been able in a number of cases to survive with less than 30 individuals for considerable periods. It is suggested that there may

be a viable population of *Nessiteras rhombopteryx*.

Reptiles must breathe air, though comparatively infrequently. A terrapin has been recorded as surviving one year of continuous submersion. Should the Nessies wish to breathe quite frequently, they would not be detected easily if the nostrils were at the topmost

point to break surface. Many accounts of head sightings speak of 'horns' or 'ears' which may be extensions of the nostrils into breathing tubes. Indeed the ancient name 'water-horse' suggests the appearance of horses' ears. With any ripple on the water it would not be difficult for a Nessie to breathe undetected. In flat calm conditions, the surface is constantly dimpled by rising fish, and again the animal would be likely to go unnoticed.

Further attempts to obtain strobe-flash or other underwater photographs with coincident sonar charts may soon lead to a more complete knowledge of the anatomy of *Nessiteras rhombopteryx*. Another technique which could produce results is sonar-linked under-

water television which could be recorded on videotape. Efforts might also be made to dredge on the bed of the loch at points where penetrating sonar may indicate more solid objects lodged in the sedimentary mud, with the possibility of finding some recent bones.

Modern concepts of conservation suggest, however, that in the case of very rare animals photographic evidence should perhaps be more frequently used by taxonomists. The ethical implications of collecting specimens of a species in danger of extinction have been raised by certain recent examples. To what extent, if any, does scientific curiosity justify risking that most irrevocable biological occurrence

—the extermination of a species? The outcry that would now be raised by any attempt to kill a Loch Ness monster for any purpose whatsoever can well be imagined.

Meanwhile tribute should be paid to all those who have worked, over many years, to identify the Nessies. They have produced a hard core of evidence indicating the general appearance of the animals. Now that their existence seems closer to being established, giving the species a name will not only provide it with the necessary protection but also focus greater attention on further studies which must in due course lead to more detailed knowledge of the animals' anatomy, biology and phylogeny. □

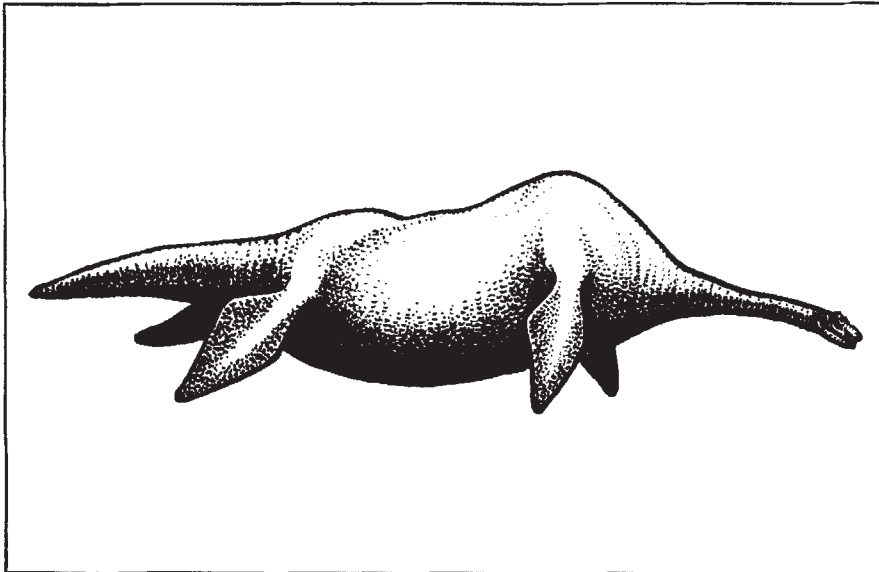


Fig. 4 Impression of the possible appearance of *Nessiteras rhombopteryx* from photographs, eyewitness accounts and sketches derived from a large number of sightings at the surface and from the accompanying underwater pictures.

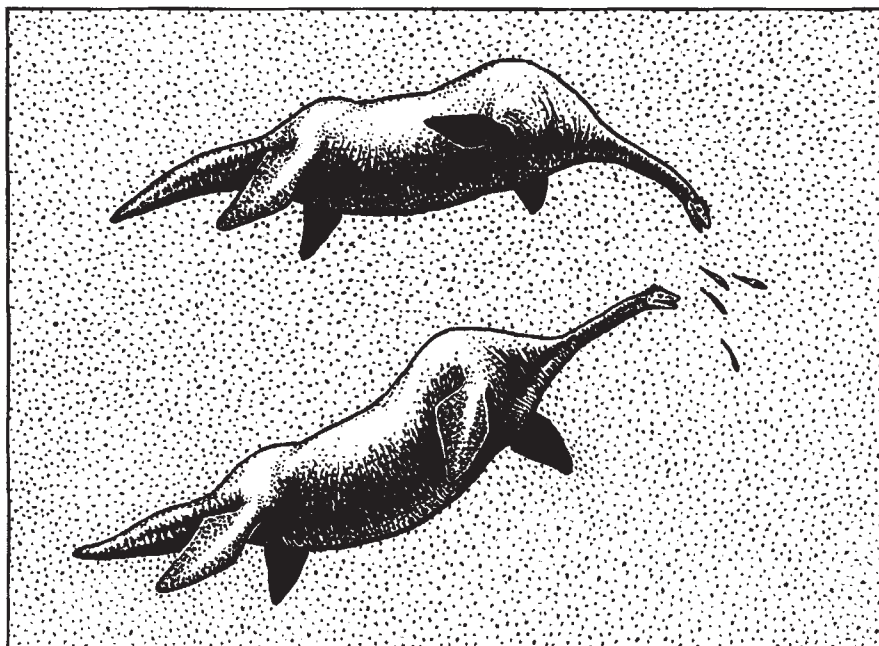


Fig. 5 The food of *Nessiteras rhombopteryx* is probably mainly fish, including salmon.

THE latest claims for the existence of the Loch Ness Monster have their origins in a series of photographs obtained by a team led by Robert Rines, a prominent Boston lawyer. Dr Rines has looked for Nessie every summer since 1970, and two sessions have yielded results. The first was in 1972, when the two 'flipper' photographs were taken underwater. Last June photographs were taken which are now being offered as evidence for the presence of the monster.

The development of two thousand underwater shots, taken automatically at regular, short intervals, revealed six frames with a discernible image, of which two are believed to show the monster. One of these, reproduced on p467, is claimed to show the head, neck and body. The other is claimed to be a close-up of the head.

Dr Rines consulted naturalist Sir Peter Scott, who has fostered an interest in the Loch Ness Monster since 1962 through the Loch Ness Information Bureau. They decided that the evidence should be presented to an invited audience of scientists at a meeting in Edinburgh on December 9 and 10 organised by the Royal Society of Edinburgh, the University of Edinburgh and Heriot-Watt University. This was to be followed by a presentation to the House of Commons.

On November 22, news of the latest claims leaked to the press. There followed a series of statements attributed to some of the scientists who had already seen the photographs. By December 1 so many people had stated their opinions in advance that the planned symposium was cancelled on the grounds that useful or impartial discussion would no longer be possible. The presentation to the House of Commons is to continue.

Microscopic blind spots

No newspaper columnist would expect to claim that 'spiders are insects' or that 'Mars is a star' without attracting a regular deluge of indignant correspondence. Yet even our most respected publications can state that typhoid is caused 'by a virus' and emerge unscathed. Brian Ford comments.

SOCIETY has a blind spot for micro-organisms. Such scientific devices as an automatic transmission system, a programmed washing machine, an electronic calculator or a sophisticated high-fidelity amplifier are familiar features of today's domestic landscape. People are equally familiar with the sights and sounds of science. The craters on Mercury or the boulders on Venus take space of their own in the press, along with integrated circuits, steel-piercing lasers and thermographs. And the hissing whistle of a transmitting satellite is as familiar to our ears as warbling whales and echoing, ghost-like bats.

The microscopic universe, however, remains obscured, and for all the undoubted familiarity of the microscope as a symbol of scientific endeavour there is ample evidence of the esoteric complexity surrounding the subject. Superstitions about infection persist a century after the popularisation of the germ theory, and though they are intimately connected with us, microbes are unfamiliar to the public.

Critics of modern microscopy argue that professional standards of routine technique are inferior too. Certainly one hears far more from research workers who claim to have a love-hate relationship with the instrument than suggestions that microscopy is enjoyable. The blind spot that exists within the discipline is revealed not only by a low average standard of practical microscopy, but also by the paucity of educational opportunities for those who wish to improve. This is vividly reflected in the way we have delayed setting up safety codes for the handling of pathogenic bacteria.

It is perhaps a legacy of nineteenth-century bacteriology that we tend to associate 'microbes' with 'bacteria', and 'bacteria' with 'disease'. It is still accepted that microbiology and bacteriology are virtually synonymous. This tradition of distaste, in might be argued, underlies our microbiological apathy, which has delayed progress in a field rich with potential.

During the debate on energy sources, for example, much was said about waves, windmills and the power of the tide. But little was heard of the solar-powered electron pump systems of algae and the capacity of micro-organisms to act as transducers. The brand of automotive fuel sold as 'Cleveland

Discol' was possibly the last one available containing fermentation alcohol, and it is somehow intriguing that the industrial liberation of metabolic energy from a 'waste' substrate is now—in an energy-seeking world—so rare.

Corynebacterium simplex is familiar as the organism which converts cortisone to prednisone and hydrocortisone to prednisolone, both more potent yet less hazardous to the patient than the parent steroid substrate. An extension of cell technology has for years offered us a range of similarly self-fuelling conversions that demand no energy supplies. Yet microbiological methods seem to be used only when an acceptable chemical-industrial approach cannot be found. Should we not reverse the process, and opt for cell technology wherever we can?

The public health field offers limitless possibilities. The antibiotic era has revealed many of the growth-regulating products of the fungi, but such familiar algae as *Microcystis* and the abundant *Chlorella* secrete bacteriostats. Little is known about the ecological importance of microbial interrelationships, yet since the 1930s it has been known that soil-borne pathogens such as *Helminthosporium* and *Ophiobolus graminis* are controlled in some way by micro-organisms in the rhizosphere. Intracellular bacteria can be found in many species of insects (including *Periplaneta*, the cockroach) which will not mature normally without them.

Some instances of overwhelming skin infections in man may be due not to the removal of commensals and the opening of a niche, but rather to the absence of specific health-promoting species—salugens (from the Greek *salus*—health) as opposed to pathogens.

Becoming microbe-conscious opens many avenues of investigation. For all our exhaustive knowledge of the behaviour of a single cell of *E. coli* or *Paramecium*, we know little about the interactions of five cells, or five hundred. The trend towards bulk *in vitro* methodologies is unlikely to resolve these issues unless it is complemented by that rare art, the microscopy of living cells.

A similar philosophical approach allows one to resolve the conflict of the 'cell-equals-egg' versus 'cell-equals-organism' debate. This involves demonstrating that a concept of func-

tional delegation can relate the specialisation of cells within a multicellular organism to the complexity of such single-celled organisms as the protozoan *Epidinium*, a rumen protein converter which possesses a brain-like motorium and a segmented skeletal plate and has an almost quasi-chordate appearance. Certainly one can argue that the loss of delegative function in malignancy allows the cancer cell to return to a less specialised, microbe-like state (and the world-wide infection of cell cultures with HeLa suggests that, even in the practical sense, that analogy holds true).

The philosophical consequences of becoming microbe-conscious range from the lighthearted concept that man is a free-swimming protozoan called a sperm, and all the complexity of an adult human is nothing more than a refined fruiting-body; to the serious and pragmatic possibility of producing crops, food and drugs from tissue culture rather than from colonies of the entire species.

But whatever the future, there can be little doubt that our orthodoxy has done a disservice to micro-organisms. The dogmatism which followed the Pasteurian view of infection must be replaced with something more realistically positive. For philosopher and technician alike, the benefits of doing so are great.

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international news

Fast-breeder urged

by Allan Piper

THE UK standing Royal Commission on Environmental Pollution, which has had the radiological safety aspects of Britain's nuclear programme under consideration since the beginning of last year, has conceded that there is a case for establishing a full-scale demonstration power station based on the controversial fast-breeder reactor (FBR). In an open letter to the Prime Minister, the commission's chairman, Sir Brian Flowers, acknowledged the importance of the FBR in Britain's energy strategy and admitted that "there are arguments for pressing ahead [with a demonstration FBR] on a reasonable time scale. To do so is to be better prepared".

Sir Brian's letter was apparently prompted by fears that the British government would reach an early decision to proceed with a commercial-scale reactor in collaboration with other European governments before the commission's investigation was complete. A demonstration FBR, "remotely sited [with] its own fuel reprocessing and fabrication plant", is seen as vital for assessing the hazards of operating a commercial fast reactor. The letter does make the point, however, that the UK need not allow itself to become finally committed to the FBR at the expense of research into other energy sources. It notes that the commission is not "yet persuaded that the energy needs of the UK in the next 30-50 years are such as unavoidably to require the deployment of FBRs on a massive scale."

Were it not for the apparent paradox of an environmental watchdog body calling for the construction of a controversial type of reactor, the publication of Sir Brian's text might have been the non-event of the year. Not only is the primary recommendation of the letter couched in predictably reserved terms, it is also little more than a reiteration of what is effectively the policy of the United Kingdom Atomic Energy Authority (UKAEA).

For some time it has been widely accepted that the next logical step in the UKAEA's development programme for fast reactors would be the construction of a demonstration reactor on a commercial scale. The prototype 250 MW reactor at Dounreay seems just about to have survived its bout of

teething problems, but the UKAEA's tentative plans for the construction of a 1,300 MW demonstration installation nonetheless involve time-scales that seem surprisingly short. The authority has talked of having a demonstration fast-breeder by the early 1980s, though the Department of Energy's decision will depend on prospects for European collaboration.

It is not altogether clear why the text of the letter should have appeared at this particular time, especially as the Prime Minister denied in his reply that an early decision on the FBR programme was expected. Sir Brian and the Prime Minister shared a platform on the day after the publication of the letter, the key passage of which highlighted "the distinction between the environmental implications of a full-scale demonstration FBR station and of a large ongoing nuclear programme". What that means is that the construction of a UKAEA demonstration reactor, which would be only nominally commercial, might be taken to signal a total commitment to the FBR, whereas the construction of the same reactor in line with the commission's recommendation can be viewed as part of an environmental appraisal. □

Protest over Sakharov

from Vera Rich, London

THE award of the 1975 Nobel Peace Prize to Academician Andrei Sakharov has inevitably drawn considerable attention to the whole problem of human rights and academic freedom in the Soviet Union. Sakharov was refused a visa to travel to Oslo to collect his prize, and a group of former Nobel winners has now issued a statement on behalf of the refusnik scientists "deprived of the freedom to work, to publish, to teach, and to travel freely, simply because they have sought to emigrate from the USSR".

They note that "we shall never regard scientific exchanges with the USSR as being easy and normal while these people are deprived of their rights . . . while arbitrary and needless bars to freedom and work are enforced and while bargains are entered into and unilaterally set aside, the existence of detente will remain pre-

carious". The "bargain" referred to is the one which the Soviet authorities made last year with Academician Veniamin Levich, whom they promised would have permission to emigrate at the end of 1975 when his security restriction expired. In October they denied that any such promise had been made.

Signatories to the statement include Professor Sir Derek Barton, Professor Sir Hans Krebs, Professor Rodney Porter, Professor Sir George Porter and Professor Max Perutz.

Meanwhile, Dr Leonid Kantorovich, joint winner of this year's Nobel Prize for Economics, arrived in Stockholm to receive his prize with the necessary exit visas and approvals demanded by the Soviet authorities. He declined to comment on their refusal to allow Academician Sakharov to travel to Oslo. □

Soviet grain estimate cropped

from Vera Rich, London

DURING this year, the estimates for the Soviet grain harvest have been consistently revised downwards from the planned yield of 215.7 million tonnes announced last spring. The final figure has, however, proved to be even lower than expected. In a statement made to the Supreme Soviet on December 3, Grigorii Vashchenko, Chairman of the Planning and Budgetary Commission, said that the average annual grain production for the ninth five-year plan (which ends this year) had exceeded that of the previous plan by 8%.

Comparative figures are a classical gambit of Soviet planners who wish to put the best possible political face on a deficit. Translated in real terms, however, Vashchenko's 8% "increase" corresponds to a total production of some 137 million tonnes, more than a quarter of the planned output. This figure, which received wide publicity in the Western media, was promptly denied by the foreign service of Moscow Radio. Moscow Radio claimed that the figures had been manipulated and that, properly understood, they reflected a "steady planned progress". In a similar way, the industrial growth rate of 4.3% for the coming year—the lowest since the Second World War, almost certainly as a result of the disastrous

harvest—was defended by the Hungarian service of Moscow Radio as marking a “very great step forward in a people's economy like the Soviet Union”.

The reason for the great deficit in grain production was attributed by Mr Vashchenko to “difficult weather conditions in several areas of the country”. This would seem to put the responsibility firmly beyond the competence of the planners, but this needs further clarification, for the expansion of Soviet agriculture, as the Virgin Lands were opened up, itself had unfavourable climatic consequences. Moreover, an assessment of the agricultural situation based on land resources, available man- and tractor-power, present capacity of irrigation channels and so forth might well reveal that, allowing a reasonable margin for losses due to adverse weather, the Soviet Union is not fully able to feed its population.

This is true of many countries, but these mostly have agreements with countries in surplus to make up the shortfall. This the Soviet government is reluctant to do, and any such negotiations—such as those with the USA—are considered “short-term” solutions to an immediate emergency. A regular deficit would throw into question the whole system of socialist planning.

In his address to the Supreme Soviet, Nikolai K. Baibakov, Chairman of the State Planning Commission, said Soviet agriculture would have at its disposal in 1976 “about 380,000 tractors, 270,000 heavy and specialised trucks, more than 97,000 harvesters, and 78.6 million tonnes of fertiliser and ‘food additives’.” Agriculture was to be considered as simply another branch of industry, and re-equipped accordingly. Similarly, Mr Brezhnev in his address noted that “in order to raise the well-being of the people, it is necessary to pursue in the future a policy of intensification in agriculture. To turn it into a highly developed branch of the economy demands the systematic consolidation of the material and technical bases of agriculture and the increasing utilisation of the economic and scientific-technical potential for this purpose. Under the Tenth Five-Year Plan, it is intended to allot greater capital investments to agriculture, and to increase supplies of equipment, machinery, and mineral fertilisers.”

This “more-of-the-same” approach seems to contrast with the hint of change contained in *Pravda* on November 14. It published a significant decree of the Central Committee of the Party, which was subsequently followed by extensive press comment. This decree approved and recommended for emulation a project undertaken by the Lithuanian Agricultural Research In-

stitute, on the improvement of the efficiency of basic research in agriculture and its implementation in practice. The obvious nature of what the Lithuanian agronomists have done makes this surprising. The project, which has lasted eight years, consisted of research into soil types, problems of land reclamation, and the productivity of various strains of pasture and human and animal feedstuffs. Soil charts were compiled, and the yield of reclaimed soils were closely examined. This raises the question of what the real basis of Soviet agricultural planning has been. Hitherto it seems to have been considered sufficient to mechanise and “chemicise” agriculture, in the hopes that a good yield must surely follow. The emphasis placed by the Party on the Lithuanian project seems to show that in spite of public pronouncements on the need to introduce industrial methods into agriculture, there is a growing awareness of the need to base all such plans on a study, not only of general climatic and geographical conditions, but also on local micro-ecologies. □

Another look at doom

The main trouble with the computer simulations of environmental change that are used for long range forecasting—such as those used by the Club of Rome to compile *Limits to Growth*—is, according to Lord Ashby, that they omit politics, taking no account of the most striking and distinctive feature of all communities of organisms: their homeostatic response and capacity for adaptation.

What is needed, he argued when he delivered the 21st Fawley Foundation Lecture in Southampton this week, is “a second look at doom”: a recognition that the prognosis may be wrong but the malady—a climacteric rather than a crisis—remains. The arrival at the second turning point in the S-shaped trends of such factors as population and consumption of resources, he said, provoked “political and even ethical responses from society”, as well as the more familiar negative feedback effects tending to reduce population or the rate at which resources are used. To ignore this, he suggested, was to fail to grasp the problem.

For Lord Ashby, a well-known biologist who was Master at Clare College, Cambridge, the essential problem was clear. He saw no guaranteed tenure for man on earth—indeed, “it would be an evolutionary anomaly if *Homo sapiens* were not to go the way of the pterodactyl”, and a “historical anomaly if western technological man's economic and social system were not

to go the way of the cultures of the Minoans and Aztecs”. But at the turning point of the S-shaped curves, values changed and deeply embedded social attitudes were reversed. This had already been revealed in UK population trends, in the consumption of energy, and even in such fields of pure science as bacterial genetics.

Exhortations to control the use of material resources and protect future generations, he therefore contended, emphasised the wrong priorities for policy-makers. The imminent danger was collapse due to political and social disintegration. Moreover, the urgent need was to do something about the communities already suffering from the perils forecast.

The main problems were neither technological nor economic, but geopolitical. With the industrialised nations dependent for some essential supplies on underdeveloped and sometimes unfriendly countries, the world was in for a succession of geopolitical confrontations, in which an alliance among nations which owned resources might be directed against nations which needed them. A shift might occur in the balance of world power to which the industrialised countries had to reconcile themselves or else face the alternative of war.

It was not only international tensions which might pre-empt the factors foreshadowing economic collapse described in *Limits to Growth*. Unresolved tensions within nations also revealed the problems stemming from what Lord Ashby described as the instability of man-made eco-systems. A pre-occupation with population control, or resource conservation, or pollution might divert attention from the industrialised nations' new dependence and their desire to maintain their liberal democratic forms—a prospect which, he argued, meant ignoring more imminent and greater dangers. □

Wilson on Blackett

PRIME MINISTER Harold Wilson was in reminiscent mood when delivering the Blackett Memorial Lecture and naming the Physics Department the Blackett Laboratory at Imperial College last week. Patrick Blackett had been professor of physics at Imperial from 1953-65, and had worked with Harold Wilson since the late 1940's, first as Wilson's appointee on the National Research Development Corporation (NRDC), on the board of which he served from 1949-64, and later as Scientific Adviser to the Ministry of Technology in the 1964 administration.

The appointment of Blackett to NRDC, Wilson said, led to one of the

most violent political reactions he had known in 30 years of Parliamentary life. Blackett's unorthodox views on world affairs, including the need to co-operate with the Soviet Union to avoid the division of the world into nuclear blocs, was the cause of much of this reaction. But he made a major contribution to NRDC, and in the late 1950's and early 60's, with Labour out of office, his experiences at the corporation had a powerful influence on his thinking about how the next Labour government might improve links between science, technology and industry. The deliberations of such as Snow, Bernal, Blackett, Carter and Bronowski helped lead to the Ministry of Technology which was in some ways NRDC 'writ large'.

It was Blackett, Wilson continued, who, when Mintech was formed, warned of the British computer industry's distress. Supportive action had to be taken within weeks, and Cabinet took a lead by reviewing all proposed departmental purchases of foreign computers. This soon cut down enthusiasm for imports, although there was probably, he added, a good PhD thesis for someone in studying how this new form of protectionism had fitted in with GATT. □

Paying for pollution

In a Hobart paper published this week by the Institute of Economic Affairs (£1), Dr Wilfred Beckerman has refined his case in favour of charges as a method for controlling pollution and reinforced his criticisms of the alternative method of governmental regulation.

Dr Beckerman, who is a member of the UK's Royal Commission on Environmental Pollution, originally made his points with Lord Zuckerman in a minority report of the Commission in 1972. He suggests that production "uses up" the environment as well as labour and raw materials. With the environment a "scarce resource", the rational objective of "optimal pollution" is to be achieved using the means accepted for the allocation of labour and raw materials.

The polluter, he contends, has to be induced to economise in "using up" the environment, and encouraged to discover means of reducing it through the price mechanism. This, Dr Beckerman suggests, would be as efficient and equitable a method as any, and would also be cheaper. In addition "cosy relationships" between polluters and officials would be circumvented. □

French research body branches out

from the Staff of La Recherche

ONE of France's major scientific research bodies, the *Centre Nationale de la Recherche Scientifique* (CNRS), has recently been taking steps to fulfill more adequately one of the roles assigned to it by its constitution, which states that it should carry out research which will be of direct benefit to the advancement of science "or to the national economy".

The institution has established an industrial relations committee to co-ordinate a policy of cooperation with industry which it has launched, and is also making strenuous efforts to encourage its researchers to consider the socioeconomic impact of their work. Alongside the traditional scientific disciplines—physics, chemistry, biology—two new branches have been created: science for the engineer, and an interdisciplinary research programme on solar energy.

In addition, the CNRS has signed agreements for the exchange of research workers with several public or semi-public companies including the oil company Elf-Erap and the French Petroleum Institute. It has also reached an agreement on scientific collaboration with the big private chemical company Rhône-Poulenc (R-P) which allows collaboration in all the major fields covered by the company or its subsidiaries. These include organic chemistry, textiles, biology, and toxicology.

Under the latter agreement, a committee with equal representation from R-P and CNRS will choose projects for joint research and organise the training of research workers. Researchers from CNRS, while retaining their salary, will be seconded for one month to the R-P laboratories, and *vice versa*. It is also hoped to involve some personnel from R-P in various committees of CNRS on which industrial representatives could serve. In order to maintain the confidentiality necessary to industry, one clause requires that "no confidential information may be passed on to a third party . . . for fifteen years". Nevertheless, researchers can publish if they have authorisation from the joint committee. If a new process should be invented through this co-operation, R-P, if it wishes, can be the sole beneficiary. Payment to the CNRS would be calculated appropriately.

The terms of the agreement, only recently made available to researchers, trades unions and journalists, have caused an outcry. This has surprised the signatories, for whom the agreement merely makes official the links

which already exist between certain CNRS laboratories and the company. They had hoped it would serve as a model for agreements with other companies: the next one is expected between the CNRS and a giant in the French chemical industry, the Péchiney-Ugine Kuhlmann company.

According to its directors, the CNRS does not commit itself to anything, since in principle every researcher is free to accept or refuse to collaborate with R-P. But for many scientists the basis of the agreement is debatable. The advantage to R-P is obvious, in that they will have the use of research workers trained and paid by the CNRS. For the CNRS, the advantage is not so clear; indeed, the agreement appears to be a gift from the public sector, not to industry as a whole, but to one privileged member. Whether or not the research workers, traditionally chary of collaborating with industry, finally feel reassured, they will have great difficulty in publishing their results, often with a delay of a year, and sometimes 15 years.

Of course, the board of directors of CNRS could be apprised of their work. How that information could be transmitted to the scientific community, or to the committees which decide promotion steps in a research worker's career, remains unclear. However, contrary to the practice in other countries, there is very little important high level research going on in industrial laboratories in France, and a period of instruction within a firm should lead, according to the thinking of the CNRS directors, to a mobility of researchers and so free CNRS posts. At present this happens only rarely.

The manner in which the agreement was made is also regarded by many as questionable. There was no consultation, or prior warning to either the commissions (made up of researchers and elected members), or to the other bodies controlling the CNRS (such as the Directorate, or the Administrative Council). But the CNRS apparently intends to push its researchers to cooperate with industry, and to reorientate its research programmes to be more directly applicable.

The idea is not a bad one in principle: recent meetings, when industrial researchers explained their problems to the more basic research workers, have shown that dialogue is possible. It is believed essential, however, that the CNRS should profit from the agreement it makes, and that it should ensure that the results of research financed by the nation are evenly shared and exploited by the nation; this is not seriously safeguarded in the contract with Rhône-Poulenc. Moreover, the more applied research must also leave room for fundamental research.

Fight for the falcon

from Wendy Barnaby, Stockholm

THE Peregrine falcon is in imminent danger of dying out in Sweden. At the beginning of this century there were an estimated 1,000 pairs, chiefly in the north, the west and the region west of Stockholm. By 1945 the number was reduced to 350 pairs, by 1965 to 35, and by 1974 to nine. This year only six pairs are left.

The falcons have declined in the past 50 years mainly because of the widespread use of pesticides, especially heavy metals such as mercury and chlorinated hydrocarbons. Along with this, hunters (particularly the owners of carrier pigeons) have systematically shot the birds and robbed their nests. The Peregrine did not become a protected bird in Sweden until 1957.

All of the information known about the plight of the falcon in Sweden has been compiled by the Swedish Society for Nature Conservation (SNF), a non-governmental, non-profit organisation with about 60,000 members. In 1972 the SNF began a project to make annual inventories of the bird and to study the reasons for its decline. This year expenses for the project amount to Sk100,000 (about \$20,000), which is provided by various private funds, the Swedish branch of the World Wildlife Fund, the Swedish Environment Protection Board and the SNF itself. As well as inventories, the programme includes studies of the falcons' food analyses of pesticides and the protection of nests and nesting areas.

The project leader, Mr Peter Lindberg, has surveyed the falcons' prey to determine the regularity with which certain species are consumed, but the results have not been very illuminating because little is so far known about the ways in which these species are

contaminated with pesticides. Nor has it been possible to bypass infected natural sources to any significant degree by releasing clean prey in the peregrines' vicinity. The problem is that the falcons spend six months every year outside Sweden, when their diet cannot be controlled.

Mr Lindberg is convinced that stable pesticides have done the birds more harm than any other single factor. Studies of the mercury content of Peregrine feathers have shown that until 1940, the mean mercury value was 2500 ng/g. In the 1940s an organic mercury preparation, alkyl-mercury, began to be used for dressing seeds to protect them against fungi. By the 1950s the falcons' mercury values had increased by ten and twenty times, reaching a peak just before the use of mercury was banned in 1966. Since then the mercury values have decreased, but in the early 1970s they were still three eight times as high as the pre-1940 mean. Not only is mercury harmful to grown birds, which become paralysed when poisoned, lose their balance and finally die, but it also reduces the chances that eggs will hatch—Peregrine eggs with a mercury content as low as 450 ng/g (dry weight) have been found unhatched.

Chlorinated hydrocarbons have also helped to reduce the falcon colony. Many of these substances are stable and are stored in the birds' fat, only to be metabolised in winter as their fat reserves are used. DDT has been widely used to protect young plants in Swedish forests until it was totally banned this year. In its broken-down form of DDE, DDT is thought to thin the eggshells, which can then crack and break more easily than normal eggs. Eggs examined in Sweden have shown that the mean thickness of the shell fell by 11.4% to 0.31 mm between 1946 and 1974.

In addition, PCB, which has been used in industry in Sweden since the 1920s, is known to disturb the body's enzyme and hormone balance, and researchers suspect it can reduce the falcon's ability to breed. There are no thorough figures on the PCB content of Swedish birds because so few have been found dead, but one suspected chain of contamination suggests that the PCB in paint used on boat hulls dissolves in the water to be ingested by sea birds later preyed on by falcons.

Since the Peregrine project began, all 568 nesting places known to have been used by the falcon in this century have been registered, and most visited. Wherever volunteers have found a pair, they have kept a 24-hour watch on the nest (sometimes using closed-circuit television cameras) over three-month periods from the laying of the first egg until the young have left the

nest. Even though falconry and the hunting of the Peregrine are banned by law in Sweden, the nests may still be disturbed. Often the culprits are foreign.

The problems of saving the Peregrine are further complicated by its migration. Calculations show that 53% of young Swedish Peregrines and 40% of older ones have been shot outside Sweden. Other countries' hunting, falconry and pesticide laws are therefore of enormous importance in the species' decline: a decline which is being registered not only in this country but in the whole of Scandinavia, Europe, North America and the eastern part of the USSR. At a conference of the International Council for Bird Preservation in Vienna last October these problems were discussed, and resolutions were passed urging that activities harmful to the falcons be banned in each country. Those to which the Peregrine mainly migrates are Denmark, England, the Netherlands, France, Belgium and northern Spain. But—as the experience of the French has shown—even if hunting is made illegal, it often takes the hunters a few years to comply.

In a last-ditch effort to save the species in Sweden, Mr Lindberg has just begun a captive breeding programme. He has gathered three falcons from Scotland and one from Sweden, and is hoping that their offspring will build up a productive stock which he will then be able to re-introduce to the wild. No quick results can be expected, though. The birds must be hatched in captivity or captured when they are very young, and no Peregrine has been known to breed in captivity before the age of five. Judging by the rate at which the natural population has been declining, there will be a sad hiatus during which no Peregrine at all will exist in the wild in Sweden. □



Threatened: the Peregrine falcon (Photo: Peter Lindberg)

Canada's Science Council reconsiders its role

from David Spurgeon, Ottawa

EVER since the creation of the Ministry of State for Science and Technology (MOSST) in 1971, the status of the older Science Council of Canada, formed in 1966, has been in some doubt. The council was formed as an advisory body to the federal government on science policy, but this was also seen as one of the ministry's roles. Now, through public pronouncements made over recent months on the rede-

finition of MOSST's role, the Science Council's position has become even less clear.

It appears that a good deal of soul-searching has been going on behind the scenes. Since a new chairman and a new executive director were appointed—both relatively young men with experience in industry—internal discussions have proceeded about the role and purpose of the Science Council, and a much more publicly visible, popular and practical orientation is envisaged.

The new executive director, John J. Shepherd, who is 46, was formerly chairman of the board of the high-technology firm Leigh Instruments of Ottawa. Josef Kates, the chairman of the council, is president of a Toronto-based firm of systems analysts. Shepherd believes that council has always tried to reach the public, mostly through its publications, but that the primary audience was other scientists, making its activities too much like "talking to ourselves".

Now the appeal will be broadened—but with particular attention being paid to communication with industry. This month, the 20 Science Council members, 7 of whom are themselves from industry, will consider a paper that has been prepared to sum-

marise the discussions already undertaken and present the views of the council's staff on the future role of the organisation. By early spring, it is hoped that agreement will have been reached on the council's purpose, some goals will have been accepted, and some decisions have been made on how to proceed to meet them.

This approach will contrast with more casual, *ad hoc* one of the past, and will, it is hoped, prevent endless debate on where the council is going. The kinds of proposals being made to the council in the paper that will be presented are these:

- The council should serve as an early-warning system to signal the need for R&D in specific areas important to the country. An international conference was sponsored by the council in Toronto in November, for example, on the subject of climatic change. Other possible topics for the future include the problem of fresh water supplies, and cold water problems for northern development.
- The council should define its role in international activities, particularly with respect to other countries' science councils.
- The council should take a view regarding some two or three important issues. One is the matter of basic re-

search, and a second the question of technological sovereignty, that is, control over the technology the country acquires and the ability to exploit it nationally. Another is the concept of what the Council calls a Conserver Society, implicit in which are recycling and growth limitation. On the question of whether it can be sold to industrialists on the board, Mr Shepherd says that the Council's approach is that it would involve a lot of industrial opportunity. "It is not a negative, no-growth philosophy but a new opportunity".

One of the questions council members will doubtless ask themselves in considering this is whether they want to take the risk of committing themselves to such a specific policy. John Shepherd hopes they will, feeling that the council has nothing to fear from a re-structured and stronger MOSST. In fact he feels that the stronger MOSST can become, the bigger the public role of the council will be, because independent, though constructive, criticism will be needed all the more. But he sees the council as faced with a challenge in reaching the public: "There is a widespread feeling that R&D is not paying off. We've got to show very clearly how science is related to jobs". □

SEATED in a meeting of the Academic Senate at Berkeley, I was listening to the annual report of the Committee on Academic Freedom. It was one of those occasions when a printed copy is distributed prior to being read from the platform. The report contained the ringing words "... in areas of intellectual, scientific, artistic endeavour it is absolutely essential to protect and, indeed, to foster the odd-ball, the deviant, the genius, that blockhead who keeps insisting against all his colleagues that the earth is round and that it circles around the sun." With magnificent courage and perfect hindsight, I thought, we rally round Galileo to thunder defiance at the Inquisitors of 340 years ago, whose muddy vesture of decay keeps them from hearing us.

But what about the genius who insists that the Earth is *flat*? Especially the one who teaches this to university students. Let us remember that for every Galileo there were hundreds of odd-balls who kept proclaiming that the Moon was made of green cheese, or that the fault, dear Brutus, was not in ourselves, but in our stars, that we are underlings! Do we fight courageously, implacably, in the name of academic freedom, or the wider cause of freedom of speech and the press, to protect and foster soothsayers and alchemists? Should the Department of Astronomy appoint a Professor of

Equal time for nonsense



THOMAS H. JUKES

Astrology because, as the resolution went on to say, "the intellectual deviant is one of our most precious resources?"

Don't overlook the fact that opponents of the scientific "establishment" are well aware of public sympathy for the underdog and of how easy it is to get equal time in the media. Some months ago I was asked to talk on

television about nutrition. When I arrived at the studio, I found that the programme was to be spiced up by having me debate a "health food" salesman who had copped the spot by demanding "equal time". He regaled the audience with an account of the difference between natural and synthetic ascorbic acids. The natural variety, said he, bent a laser beam of light to the right, but synthetic ascorbic acid turned it to the left. Of course, I told the audience that it ain't so, but it was another case of disagreement between experts, and the television station loved it. Nothing like a one-against-one controversy to please listeners and boost the ratings.

So ended my effort on this occasion to bring nutrition from the ivory tower to the public ear. I departed, still dedicated to free speech, but well aware that those who own and control television stations and newspapers decide who gets it. And, right now, the odd-balls are doing very nicely. Acupuncture today, moxibustion tomorrow. Horoscopes were cast in a *Nature* article in April last year. We are approaching a state of affairs in which reactionary deviants who insist that the Earth circles round the Sun may indeed need protection.

Perhaps the Committee on Academic Freedom was right without really knowing why.

correspondence

Missing academicians

SIR,—The collective letter of 72 members of the USSR Academy of Sciences condemning the activity and stands of Andrei Sakharov (*Izvestia*, October 26) is worth analysis. According to *World of Learning* (1974–75 edition), the academy has 236 full and 445 corresponding members, that is, 671 in total. This means that only about 10% of the academy's members put their signatures on the anti-Sakharov statement.

The list of signatures does not contain the majority of the most famous academicians—physicists, who no doubt know better than others Sakharov's scientific and moral qualities. Among those physicists who did not sign the statement were P. L. Kapitsa, Bogolyubov, Migdal, Lifshitz, Zeldovich, Pontecorvo, Leontovich, Ginzburg, Budker, Belyaev, Frank, Cherenkov, Kadomtsev, Khariton, Kikoin, Linnik, Sagdeev and Vernov.

Similarly, we could not find among the 72 academicians the signatures of such leading Soviet mathematicians as Kolmogorov, P. S. Alexandrov, A. D. Alexandrov, Petrovskij, Pontryagin, Sobolev, Vinogradov, Kantorovich, Gelfand and Shafarevich.

In addition, the statement was not signed by T. Lysenko and M. Sholokhov. All told, the anti-Sakharov statement is much more a testimony concerning the real situation and moods in the Soviet Academy of Sciences than a simple condemnation of Andrei Sakharov.

F. JANOUCH

Research Institute for Physics,
Stockholm

Crisis in Italian universities

SIR,—The article by Gillian Boucher entitled "Continuing crisis in Italian Universities" (November 20, p190) should, I feel, be completed by a few comments to give a fair representation of the situation. The comments which follow are based mainly on the situation in most of the Departments of Physics in Italy (which I do know from personal experience) but can apply with minor changes to almost all the faculties; the problems of Law, Engineering and Medical Sciences are similar in some respects, but different in others.

The central point of the article, namely that the Italian universities are

in a state of agony, is correct, and this may, after all, be the only fact of general interest to your readers. It is also true that, due to a set of demagogic laws, any student can rather easily get his final degree; this is not stated explicitly in the article, but can perhaps be inferred.

What is omitted altogether is the fact that the demagogic laws mentioned above achieved the intended aim of introducing into practically permanent staff positions a large number of people with no scientific qualification but disposed to aggressive political activity (95% or more of them are leftists). This is the main reason why, in spite of the present political turbulence, the invasion of departments is much less frequent than it used to be a few years ago: those who invaded or stimulated the invasions now have permanent positions inside.

This process, in addition to depressing the average scientific level in many departments to unbelievably low values, has filled each department with large numbers of "teachers" or post-doctoral "research workers". They teach or do research only in Marxism, or, to be generous, in some cases, in Science and Society. To give an example, the number of staff members with an office in the Department of Physics in Genoa is ninety-five; the total number of students in Physics, covering all the four years of the curriculum, is approximately the same. Some teachers do not have any students at their courses.

This overcrowding of "democratic" but ignorant staff members in the departments has three important consequences:

- those people who have obtained their positions fighting against "meritocracy" continue to dedicate all their activity to similar issues;
- they reproduce themselves;
- they occupy, for ever, a large number of positions.

In these conditions no brilliant young man has a chance of finding even a temporary position in the university.

These remarks show, I hope, that the main problems of the Italian universities are not of money (which is, however, badly administered), nor the fact that the full professors try to conserve their privileges (as one of the privileged I would like very much to know from your correspondent what my privileges are). The main problems are the demagogic and antimeritocratic edicts

which were strongly supported for many years by the *Corriere della Sera* (to which Gillian Boucher refers). It is a well known tactic of disruption to destroy something first and then to complain about how badly it works.

GIACOMO MORPURGO

Department of Physics,
University of Genoa, Italy

Kennedy's deed

SIR,—You include a comment (November 6, p5) entitled "Kennedy's good deed" regarding the U.S. Senate vote to ban the use of diethylstilboestrol as cattle feed supplement. A number of controversial matters are introduced into this short article, but I wish to protest the attribution of purely political motives to Senator Kennedy's sponsorship of this bill. Those of us who know of Senator Kennedy's past efforts on behalf of cancer research in this country must consider these remarks both unfair and uncalled for. One would hope for much higher standards from *Nature*.

GEORGE P. STUDZINSKI

New Jersey Medical School,
Newark, New Jersey

Stockport to Darlington?

SIR,—I have just seen your issue of September 25, in which you quote from an 1875 *Nature* in *A hundred years ago*. The extract deals with the opening of the first railway, but refers to the line "between Stockport and Darlington". Is this a 100-year-old misprint, or one perpetrated as recently as September 1975?

I am reliably informed that it is not even today possible to go from Stockport to Darlington direct: the trip necessitates two changes of train and takes 3–4 hours. The distance between Stockton and Darlington, which gave Stephenson's railway its correct name, is 30 miles or so.

B. JOVE

Hove, UK

Unfortunately the error was made in 1875, and has been faithfully reproduced! —Ed.

Box and Cox

SIR,—Cox (August 14, p524) writing on Box! You're having us on.

R. S. BRAY

Medical Research Council,
The Gambia

news and views

Low grade fuel: burning problems solved?

from Allan N. Hayhurst

IN a recent article (*Nature*, **257**, 367; 1975) Lloyd and Weinberg of Imperial College, London, point to the immense waste of energy by the discharge of low grade fuels to the atmosphere. They quote as common examples the direct venting to the air of upcast gases from coal mines, of methane from sewage or wastes fermenting in air and also of the wide variety of exhaust gases from a great range of industrial processes. It has been argued, for instance, that all the machinery of the British coal industry could be powered by burning the methane present in the ventilation air from mines. Also, large industrial flares appear to everyone as a common and puzzling waste of good fuel; and of course, all this is to say nothing of the pollution associated with some of these fairly frequent practices. Lloyd and Weinberg also feel strongly on the point that until recently many combustion devices have been very simple because often there has been little incentive to engineer or design more efficient plant. The potential savings are clear, for they maintain that an improvement in the use of energy from combustion systems of only 12.5% could, at this moment, replace all other present sources of power, including nuclear.

Another aspect of the picture is that it seems certain that in the future prime hydrocarbon fuels will increasingly have to go to the chemical industry for processing, because in terms of world resources they are or will become too precious to burn. There will be an increasing need to burn only low grade fuels, which must include domestic and industrial waste. One great difficulty here is that methane, say, will not burn if diluted with air

to more than twenty times its own volume. This is the problem to which Lloyd and Weinberg address themselves, namely that of devising a way of burning very lean gas mixtures, which are normally well beyond the limits of flammability.

Their interesting contribution is to construct a burner with a cylindrical geometry, which in cross section looks like a swiss roll. The mixture of air and fuel spirals in an elongated channel from the periphery towards a combustion chamber on the axis of the arrangement, while the products of combustion emerge from the middle in the space between adjacent layers of entering fuel and air. The purpose of such a configuration is to provide a large recirculation of heat, so that an appreciable fraction of the energy released by combustion is transferred back to the entering feed gases. By minimising in this way the loss of energy from the burner at the centre, the temperature there is forced to rise to levels much higher than would be obtained without heat recirculation. This 'amplification' of the temperature by concentrating heat in the combustion chamber facilitates the stable burning of very lean mixtures of fuel. For instance, in a small scale investigation at Imperial College, a mixture of 1% of methane and air was burnt to give a temperature of 1,400 K, when normally the flammability limit is 5.3% of methane in air. Further improvements seem possible by increasing the extent to which heat is recycled and also by providing extra lagging for the entire arrangement. It should be noted that nothing particularly special has been done to the burner, which can be an ordinary combustion chamber or

even a fluidised bed. Certainly one effect of these findings is to extend the idea of a fuel to almost anything that can generate heat, no matter how small in amount compared with that from conventional high grade fuels. Lloyd and Weinberg thus envisage this as enabling us to extract the energy from those unburnable waste gases and also to minimise some of our air pollution at the same time. In addition, they mention the possibility of humidifying, heating and sterilising the air in hospitals by removing it to burn small quantities of hydrogen in it.

One important consideration of these devices is the efficiency with which energy can be taken from them and converted into, for example, mechanical work in an engine. On this question of thermodynamic efficiency the authors conclude, at first sight possibly surprisingly, that the conversion of the heat of combustion into other forms of energy can be achieved with a greater efficiency if the fuel is first diluted with excess air and then burnt in this new way. In fact, heat recirculation appears to increase the potential (or Carnot) efficiency from 60 to around 77% for a flame temperature of 1,500 K and herein lies much of the attractiveness of the device. In their extensive consideration of all the possible uses of the swiss roll burner, mention is also made of heating some other fluid, which, for example, undergoes an endothermic chemical reaction at high temperatures. Clearly they have in mind the production of a higher grade fuel from a low one, with the water-gas reaction or reforming as possibilities.

These burners based on large heat recirculation have been described

before (*Nature*, **233**, 239; 1971 and **251**, 47; 1974) by Professor Weinberg. A very strong case has now been presented for quickly taking their development a stage further, by for example, building larger versions to discover the problems of operating on a practical scale. For example, will start up be a problem after what is in effect a burner in a heat exchanger, has been scaled up? Also, just how economic will they be?

So far only gaseous fuels have been investigated in these laboratory experiments and clearly extension to solid and liquid ones is worth considering. Two solid fuels—coal and refuse—spring immediately to mind and having established a certain degree of feasibility with methane, it is important to study various possibilities for recycling heat in these cases. In addition, the manufacturers of conventional combustion equipment, whether it be of large industrial furnaces, small scale domestic burners, internal combustion

engines, rocket motors or jet engines, will have to look at heat recirculation to see how some or much more of it can enable them both to burn lower grade fuels or increase the efficiency with which energy is used. One imagines that there might well be considerable opportunities for improving the performance of existing plant. Pollution will always be an important factor whenever fuels are burnt, and here there are real possibilities of reducing the amounts of carbon monoxide, unburnt hydrocarbons and nitric oxides discharged to the atmosphere. One problem with building up very large temperatures in a combustion chamber is the perennial one that the containing walls must neither melt nor oxidise. Even so, this new approach to combustion engineering, backed by some very telling work in the laboratory, offers immense potential to make a better use of scarce resources in a remarkably varied set of circumstances and conditions. □

Control of enzyme synthesis by steroid hormones

from Robert Shields

ALTHOUGH it is now clear that the basic control of gene expression in prokaryotes is at the level of gene transcription it is still a cliché to say that control in higher organisms is "less well understood". One mammalian system that has received a great deal of attention (especially in the laboratory of the late Gordon Tomkins) is the control of tyrosine amino transferase (TAT) synthesis by steroid hormones.

When glucocorticoids are added to a line of rat hepatoma cells (HTC cells) the rate of *de novo* synthesis of TAT increases. This increase presumably occurs through increases in the level of TAT mRNAs, as inhibitors of RNA synthesis such as actinomycin D (Act D) will prevent induction and steroids do not regulate TAT synthesis at the translational level (Scott *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **69**, 2937; 1972). If the hormone is washed out of the cultures the level of the enzyme falls (termed deinduction), but surprisingly, if Act D is added at the same time, the enzyme levels do not fall and may even increase (superinduction). On the basis of these observations Tomkins and his collaborators suggested that TAT synthesis was governed by two genes, one being the structural gene for TAT mRNA, and the other coding for a labile post-transcriptional repressor which destroys

TAT mRNA (Tomkins *et al.*, *Science*, **166**, 1474; 1969).

In the original model steroids were envisaged acting post transcriptionally by interfering with the action of the repressor and leading to its destruction, thus allowing TAT mRNA to accumulate. When the inducing steroid is removed, the model predicts that repressor levels will rise and TAT mRNA will be destroyed. The maintenance of TAT levels in the presence of Act D (even after steroid removal) was explained by the drug inhibiting repressor synthesis, resulting in the labile repressor disappearing and TAT synthesis continuing unimpeded. Unfortunately it has not yet proved possible to quantify TAT mRNA directly, so its levels are inferred indirectly from the rates of TAT synthesis (Steinberg *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **72**, 2007; 1975).

The original evidence for post-transcriptional regulation depended heavily on the maintenance of TAT levels by Act D after inducer removal. Act D might stabilise TAT mRNA (as required by the model), or more prosaically, it might interfere with TAT degradation and so maintain high levels of the enzyme. This last point has been the source of some controversy. When HTC cells are growing rapidly the half life of TAT degradation is about 3 h

and is unaffected by Act D (Tomkins *et al.*, *Nature new Biol.*, **239**, 9; 1972). If the cells are not growing because of nutritional deprivation, however, this rate is doubled and is slowed by Act D (Auricchio *et al.*, *Nature*, **224**, 806; 1969). These results showed that the maintenance of TAT levels by Act D in growing cells was not due to inhibition of TAT degradation. Exactly the opposite interpretation was reached using another hepatoma cell line grown in monolayer culture (Kenney *et al.*, *Nature new Biol.*, **246**, 208; 1973). These workers produced evidence to show that in their experimental conditions Act D maintains TAT levels by interfering with TAT degradation. The rates they observed, however, were equivalent to the enhanced rate seen in non-growing HTC cells. If the cells used by Kenney were not proliferating rapidly, the results from the two groups are then not necessarily incompatible, Act D only affecting the rapid rate of degradation seen in stationary cells.

If the levels of TAT remain constant in the presence of Act D, then it follows that the rate of enzyme synthesis must be balanced by its rate of degradation. This rate is estimated by incorporation of radioactive amino acids into immunoprecipitable enzyme during a short pulse of label. Once again the results from different groups are conflicting—Tomkins and his co-workers reporting that Act D hardly affected TAT synthetic rates (Tomkins *et al.*, *Nature new Biol.*, **239**, 9; 1972), while results from another group reported that Act D inhibited TAT synthesis (Kenney *et al. op cit.*). Again the discrepancies can be explained by a careful scrutiny of the experimental procedures. Tomkins and his group express their result as 'relative rate' of TAT synthesis, that is, incorporation of radioactivity into immunoprecipitable TAT as a proportion of the total incorporation into cell protein. Kenney's group expresses its results as radioactivity incorporated in TAT. A careful study of the rates of TAT synthesis at various times after inducer removal and after addition of Act D reconciles these observations (Steinberg *et al.*, *Cell*, **5**, 29; 1975). Whichever way the results are expressed it is clear that Act D will stabilise TAT synthetic rate (and by inference TAT mRNA) against the rapid decline of synthetic rate seen after inducer removal. This stabilisation is more dramatic when expressed on a relative rather than an absolute basis. These results suggest that TAT mRNA is stabilised relative to other mRNAs by the action of Act D and is consistent with the idea of a post-transcriptional repressor governing TAT mRNA stability. If this interpretation is correct other inhibitors of RNA synthesis

should also stabilise TAT levels after the inducing steroid is removed. Several inhibitors have been tested with different results. One, MPB, will maintain TAT levels and has no effect on enzyme degradation suggesting that TAT mRNA is stabilised by the drug (Levinson *et al.*, *J. biol. Chem.*, **246**, 6297; 1971). Other inhibitors such as cordycepin and camptothecin fail to maintain enzyme levels unless Act D is also present (Dethlefsen, *J. cell. Physiol.*, **86**, 155; 1975; Bushnell *et al.*, *Biochem. biophys. Res. Commun.*, **56**, 815; 1974). Both cordycepin and camptothecin affect protein as well as RNA synthesis, however, and so a decline in enzyme levels could mean that the inhibitors are affecting protein synthesis rather more than they are stabilising mRNAs. It is not possible to resolve this unambiguously without a more direct assay of TAT mRNA levels. Fortunately, not all the evidence for post-transcriptional regulation of TAT mRNA levels relies on inhibitors. When induced HTC cells are enucleated and the steroid removed, TAT levels remain high in the face of normal enzyme degradation (Ivarie *et al.*, *J. cell. Physiol.*, **85**, 357; 1975). This strongly suggests that a nuclear function is required for the inactivation of TAT mRNA. The results of the Act D experiment suggest that this may be transcription of the post-transcriptional repressor gene.

If the post-transcriptional repressor exists, then steroid may regulate TAT mRNA accumulation either by interfering with repressor synthesis and/or action and so altering the rate of destruction of TAT mRNA, or by acting directly on control elements governing mRNA production. The kinetics of changes in mRNA levels on induction and deinduction would be different in the two models. Using rates of TAT synthesis as an indirect measure of TAT mRNA levels it is possible to fit the data to either model; however the model whereby steroids govern mRNA production rather than destruction seems to be rather more plausible and requires more reasonable assumptions (Steinberg *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **72**, 2007; 1975). The results do not define the point at which steroids act on mRNA production, but the relatively long lag between nuclear localisation of the steroid and the appearance of functional TAT mRNA might suggest that steroids do not govern gene transcription but some later stages in the processing of TAT mRNA precursors.

The picture that emerges from the work of Tomkins and his group is that eukaryotes may regulate the synthesis of certain proteins by regulating the levels of their respective mRNAs. This can be done not only by altering their

rates of production but also by affecting the rates of destruction of these mRNAs. There is now abundant (admittedly indirect) evidence to suggest that many diverse cell types also exhibit a similar type of regulation (Tomkins *et al.*, *Nature new Biol.*, **239**, 9; 1972). □

Proteins in and out of membranes

from a Correspondent

THE current model of a biological membrane is quite complex. The lipid and protein are seen to be organised as an intimate mosaic in which some of the protein is loosely attached to the surface of the phospholipid bilayer, some partly buried in the hydrophobic core of the bilayer and some spanning the bilayer. Yet this model, which has gained general acceptance over the past decade, was preceded by a much simpler one, in which all the protein was seen to reside on the polar surfaces of the lipid bilayer, and which, for nearly thirty years, stood without being seriously challenged. When this simple model was replaced, it was not because the localisation of a protein within the lipid bilayer had been directly demonstrated but because it became difficult to reconcile the model with such overall physical properties of membranes as their optical rotatory dispersion spectra or such dynamic properties as active transport. This situation reflected the lack of reliable methods of determining the localisation of membrane proteins.

The elegant experiments of Henderson and Unwin (*Nature*, **257**, 28; 1975) on the photon-driven proton pump in the membrane of *Halobacterium halobium* have now provided direct and unequivocal evidence of a protein spanning the hydrophobic core of a membrane. Most workers in this field will, however, read this work with admiration but then return to their own membranes the proteins of which cannot be induced, either individually or collectively, to form the regular lattice needed for resolution by the method used by Henderson and Unwin. It is therefore appropriate that two papers should have appeared in the past few weeks that highlight the strengths, the difficulties and the weaknesses of some of the classical methods and which are also the latest results of two series of experiments that have led to the construction of a model for

the membrane localisation of the glycoprotein, glycophorin. Glycophorin is a heavily glycosylated protein that can be isolated from human erythrocyte membrane. It carries several biological activities including the MN-blood group activity. Tomita and Marchesi (*Proc. natn. Acad. Sci. U.S.A.*, **72**, 2964; 1975) have now published the complete amino-acid sequence and the oligosaccharide attachment sites of the protein. It consists of three distinct domains, two polar ones at the N and C-terminal ends and a non-polar segment in the centre. The sixteen oligosaccharides are all linked to the N-terminal polar domain, seven to serine and eight to threonine hydroxyl groups. Marchesi and his coworkers have suggested that the non-polar segment spans the hydrophobic core of the erythrocyte membrane leaving the N-terminal domain exposed on the outer surface of the membrane and the C-terminal domain on the inner.

The first worker to suggest that the protein did span the membrane was Bretscher. He treated intact red cells and ghosts with the vectorial probe, formyl-³⁵S-methionyl sulphone methyl phosphate (FMMP). This probe will not penetrate the membrane of an intact cell and thus labels only the protein exposed on the outer surface. Bretscher found that the glycophorin isolated from the ghosts was more heavily labelled than that from the intact cells and assumed that the ghost membrane was more permeable to the probe and that exposed protein on the inner surface had been labelled. His latest paper (*J. molec. Biol.*, **99**, 831; 1975) identifying the internally labelled fragments with those derived from the C-terminal end by the sequence workers greatly strengthens his assumption and the model.

The value of both sequence work and vectorial probe experiments is demonstrated by the result, and the difficulties, especially those found in subjecting heavily glycosylated proteins to standard analytical techniques, have been well described by both authors. The papers also illustrate at least one pitfall. Marchesi's group had presented further evidence for the internally exposed domain in the lactoperoxidase labelling pattern obtained for glycophorin. It resembled the FMMP pattern in that the protein was more heavily labelled in ghosts than in intact cells. Lactoperoxidase is said to label only tyrosine and histidine but both the complete sequence work and Bretscher's amino-acid analysis of the C-terminal fragments show that these residues are absent from this segment of the protein. Clearly one must know the precise specificity of a probe and the permeability of the membrane to it if it is to be of any use.

The biological activities of glycoporphin, such as blood group activity and influenza virus binding capacity, do not immediately indicate the role of this protein in the membrane and the above model does not help much in this respect. The intramembraneous segment may simply be an anchor or possibly provides a channel for ion transport, though this function is usually ascribed to the other major glycoprotein of the red cell membrane. The predominance of adjacent threonine and serine links to the carbohydrate is unusual and in this superficial way the N-terminal domain has affinities with the gastric mucus glycopeptides. □

Polarised light and insect vision

from our *Insect Physiology Correspondent*

MORE than a quarter of a century ago von Frisch announced to a sceptical world that honeybees could orientate themselves by the pattern of polarised light in the blue sky. It was soon found that ants could do the same; that even insect larvae with simple ocelli had the same ability; and that crustacea living in the dim illumination of the sea floor were exceptionally sensitive to changes in the plane of polarised light. For a time it was argued by some authors that polarised light was not perceived directly but only after reflection from a dark background; but it is now well established that that is not necessarily so. The plane of polarisation can be perceived directly by the compound eye, but the mechanism of such sensitivity has remained a matter of continuing controversy.

It has been established that this perception has nothing to do with the structure of the cuticular cornea, nor with the crystalline lens; the analyser is clearly in the rhabdomeres, the retinal receptors. What has remained uncertain is whether it is the geometry of folding in the membrane of the rhabdomere that is responsible for a 'form dichroism', or whether, as in Polaroid, the molecules of the receptor pigment are themselves anisotropic or are so oriented as to provide an 'intrinsic dichroism'.

A recent paper by A. W. Snyder and S. B. Laughlin (*J. comp. Physiol.*, **100**, 101; 1975) goes far to clarify this problem. As with so many controversies both parties share the truth: the relative importance of form dichroism and intrinsic dichroism varies in different eyes. The eyes of vertebrates are not at all sensitive to changes in the plane

of polarisation; but when the outer segment disks of the rods in the vertebrate retina are observed from the side, it is clear that they contain absorbing dipoles which are oriented at random in the plane of the membrane. The same applies to the microvillus membrane of the separated rhabdomeres of flies. On the other hand, in the fused rhabdoms of crustacea the dipoles are highly aligned along the axis of the microvillus membrane. It may well be that the rhodopsin molecule is asymmetric in crustacea and spherical in the fly microvilli.

The authors show that the amount of light absorbed by a photoreceptor depends on the orientation of the absorbing dipoles within its membranes: there is an optimum orientation for the photoreceptor to have a maximum absorption of unpolarised light, and this optimum varies with the organisation of the photoreceptor. In the vertebrate outer segment, maximum sensitivity is given by a random orientation in the plane of the membrane. In the unfused rhabdoms of flies the optimum sensitivity is again given by a random orientation of dipoles in the plane of the microvillus—and this gives a low dichroic ratio of about 1.7 to 1. In the fused rhabdoms of crustacea and of worker honeybees the dipoles must be highly aligned with the microvillus axis to provide the rhabdom with maximum absorption of unpolarised light; and this may yield a dichroism as high as 20 to 1.

Thus it would seem that the dichroism of the receptor membrane is merely a consequence, or by-product, of an adaptation which will provide the photoreceptors with optimum sensitivity to unpolarised light by maximising the effectiveness of every available rhodopsin molecule. The honeybee has utilised the bonus of polarisation sensitivity for practical purposes, in the orientation of its flights when the Sun is excluded from the field of view.

Inverse alpha decay

from P. E. Hodgson

THE spontaneous emission of alpha particles from radioactive nuclei has been studied since the earliest days of nuclear physics, and indeed Rutherford deduced the existence of the nucleus itself from the results of his experiments on the scattering by thin metallic foils of alpha particles emitted from natural radioactive sources.

It was soon found that the half-lives of radioactive nuclei, the times in which the intensity of alpha particles from a particular source drops to half its

initial value, vary over a wide range from $0.3 \mu\text{s}$ to about 10^{17} yr. This remarkable range was explained quantum-mechanically by Gamow as due to the sensitivity of the rate of tunnelling through the nuclear potential barrier, of the wavefunction representing the alpha particle to the width of the barrier. With a reasonable model for the potential the experimental range of half-lives could be reproduced, and this was one of the first successful applications of quantum mechanics to the nucleus.

In subsequent years the theory of alpha decay has been developed in detail, and it has been found possible to express the probability of alpha emission as the product of the probability of formation of an alpha particle on the nuclear surface and the probability that it will penetrate the nuclear potential barrier. This enabled the relative values of a large number of alpha decay probabilities (related to the half-lives) to be calculated with good accuracy, but it was not possible to reproduce the absolute values because of the complexity of the nuclear structure calculation needed to find the alpha formation probabilities and the extreme sensitivity of the penetration probability to the details of the nuclear potential.

Nuclear structure calculations are easier to carry out for light nuclei because they have fewer nucleons, and there are several excited states of light nuclei that can decay by alpha emission, and this provides more favourable conditions for testing theories of alpha decay. Detailed calculations of the alpha decay of some excited states of ^{20}Ne by Arima and Yoshida (*Phys. Lett.*, **40B**, 15; 1974) successfully gave not only the relative but also the absolute alpha decay rates in good agreement with the experimental data.

Another interesting way of testing theories of alpha decay is by studying the inverse process of alpha capture, and this has been made possible by recent studies of alpha transfer reaction (*Nature*, **252**, 270; 1974; **255**, 373; 1975). The main difficulty is that the cross sections of alpha-transfer reactions fall rapidly for heavier nuclei, but this has now been overcome by a group working at Rochester (De Vries *et al.*, *Phys. Rev. Lett.*, **35**, 835; 1975).

They chose the (^{16}O , ^{12}C) reaction as it generally has a higher cross section than other alpha-transfer reactions, and measured differential cross sections of the $^{208}\text{Pb}(^{16}\text{O}, ^{12}\text{C})^{212}\text{Po}$ reaction at 93 MeV to the ground and $0.727 \text{ MeV } 2^+$ states of ^{212}Po . These cross sections have the bell-shape characteristic of peripheral transfer reactions, and they were well fitted by finite range distorted wave calculations taking account of the nuclear recoil. The

transferred alpha particle was treated as a single particle and no account was taken of its internal structure; this is called the cluster approximation. This analysis gave a ratio of spectroscopic amplitudes for the two reactions of $S(0.727 \text{ MeV } 2^+)/S(\text{g.s.}) = 0.64 \pm 0.13$. Only the ratio could be obtained as the cluster approximation introduces unknown uncertainties in the absolute value of the calculated cross section.

The nucleus ^{212}Po also decays radioactively, and the analysis of the half-lives for alpha emission gives comparable spectroscopic information. Several different potentials were used to calculate the barrier penetration probabilities, and although the absolute values differed considerably they gave a consistent value of the ratio of spectroscopic amplitudes for the same two processes of 0.61 ± 0.24 .

Although they are still subject to considerable uncertainties, these two values of the ratio of the spectroscopic amplitudes from alpha transfer and alpha decay are certainly consistent, and provide good overall confirmation of the two theories used to obtain them. In particular, the agreement supports the interpretation of the alpha-transfer reaction as a single one-step process that can be treated by the cluster approximation. $\square$

Aspirin's target in anti-inflammatory action

from R. J. Flower

THE multi-enzyme complex which synthesises prostaglandins (PGs) ("PG synthetase", "fatty acid cyclo-oxygenase") is bound firmly to the "microsomal" membranes of cells, a fact which has naturally proved very frustrating both to enzymologists and pharmacologists who would like to study pure preparations of this unique dioxygenase. A definite advance was made last year when three Japanese workers (Miyamoto, Yamamoto and Hayaishi, *Proc. natn. Acad. Sci. U.S.A.*, **71**, 3645; 1974) succeeded in solubilising the enzyme and resolving it into separate "oxygenase" (catalysing the transformation of endoperoxide intermediates) and "isomerase" (catalysing the transformation of endoperoxides to PGE_2) components.

Now another step forward has been made by a group at the Washington University School of Medicine who have further characterised this enzyme and reported that the catalytic unit is a protein of molecular weight 85,000. To accomplish this, Roth, Stanford and Majerus (*Proc. natn. Acad. Sci.*

U.S.A., **72**, 3073; 1975) used an ingenious adaptation of the finding that aspirin (acetylsalicylic acid) and its congeners inhibit PG synthetase (Vane, *Nature new Biol.*, **231**, 232; 1971; Smith and Willis, *ibid.*, 235; Ferreira *et al.*, *ibid.*, 237). Although the detailed mechanism of aspirin inhibition of the enzyme was unknown, the Washington University group used as a working hypothesis the known ability of the drug to acetylate proteins. After synthesising aspirin with a tritium label on the labile acetyl group, they incubated the drug with crude PG synthetase preparations derived from human platelets, and sheep and ox seminal vesicles. After incubation the enzyme was solubilised in sodium dodecyl sulphate and subjected to polyacrylamide gel electrophoresis. The bulk of the radioactivity in each of the tissue digests was found to migrate as a protein of molecular weight 85,000. No incorporation of radioactivity was found when the aspirin was labelled in the aromatic ring. Evidence that PG synthetase (and not other microsomal proteins) was specifically acetylated came from the good correlation between the low concentrations of aspirin required for acetylation and enzyme inhibition and the rapid time taken for both reactions. The authors' claim was further substantiated when it was found that protein acetylation was blocked by the fatty acid substrates of the enzyme (arachidonic and dihomogamma-linolenic acid) as well as by another potent PG synthetase inhibitor, indomethacin. Aspirin is thought to block the initial dioxygenase reaction and it would thus seem that the catalytic unit (or subunit) of the dioxygenase enzyme has a molecular weight of about 85,000, and that acetylation of the active site is the mechanism of aspirin inhibition—although as the authors are careful to point out, aspirin binding at a distant "allosteric" site could also prevent arachidonic acid binding at the true catalytic site.

How will this finding increase our understanding of PG synthetase? As well as contributing an important piece of information about the enzyme it suggests many other fascinating experiments: the acetylated protein could itself be digested and the labelled amino acid identified. This would be helpful in building up a picture of the enzyme's catalytic centre, and ultimately aiding the design of novel anti-inflammatory agents. Pharmacologists will undoubtedly want to know whether any other "aspirin-like" drugs act in an analogous fashion, and perhaps the specific labelling of the enzyme with aspirin will help solve the vexed question—in which membrane fraction is PG synthetase actually located?

Because of the great diversity of

structures amongst the aspirin-like drugs (see Flower, *Pharmac. Rev.*, **26**, 33; 1974) it would seem unlikely that they could all work by way of the mechanism which Roth and his colleagues propose. One obvious exception is salicylic acid itself which lacks acetylating capacity, and which *in vivo* (though not *in vitro*) is equally effective in inhibiting prostaglandin synthesis (Hamberg, *Biochem. biophys. Res. Commun.*, **49**, 720; 1972), although there are differences in its latency of action. Coming as it does, however, in the midst of so many new developments in the PG area, this finding by the Washington University group could prove to be a timely stimulus to further research on the enzyme complex itself.

Christmas trees

from Peter D. Moore

As yuletide approaches, a botanist's mind inevitably turns to thoughts of the gymnosperms and their symbolic significance to man. From early prehistoric times certain members of this group, including that Christmas favourite, the spruce *Picea abies*, seem to have had their fortunes closely linked with the activity of man. For example, Markgraf (*Nature*, **228**, 249; 1970) has suggested, on the evidence of numerous radiocarbon dated pollen diagrams, that the spread of spruce in Switzerland occurred at different times in different areas, but was frequently accompanied by evidence of human disturbance of the forest, such as weed and cereal pollen. She felt that prior to disturbance the spruce was unable to invade the stable forests of *Abies alba*, *Pinus cembra* and the mixed deciduous forests.

Moe (*Bot. Notiser*, **123**, 61; 1970) has also shown that the spread of spruce in Fennoscandia was slow. It arrived in eastern Finland around 3,000 BC, but had still not penetrated far into Sweden by 500 BC. But Tallantire (*Nature*, **236**, 64; 1972; *Norweg. J. Bot.*, **19**, 1; 1972) does not regard man as the primary cause of its western migration in Fennoscandia. He believes that the distribution of spruce is essentially climatically determined, the critical factors being high summer and low winter temperatures. The spread of spruce, he claims, was a stepwise process, each advance corresponding to a period of suitable climate. He is prepared to admit that once established in an area its subsequent spread and population expansion in the locality could be assisted by human clearance of virgin forest. Thus, from microclimatically and edaphically favourable nuclei, spruce could attain a wide-



A hundred years ago

MR. CASELLA, the well-known scientific instrument-maker, has sent us a specimen of a compass which will be a great boon to the many who are ignorant of the difference between the magnetic and the geographical poles, and of the fact that an ordinary compass points to the former and not to the latter, the difference in this country at present being about 19° . The great advantage of Mr. Casella's "unmistakable true north compass," is that it points to the true or geographical north, being corrected for use in the United Kingdom, and capable of adaptation to any locality in any part of the world. It is a card compass of beautiful workmanship, swings with perfect ease, and by means of a black cone on a white ground, the merest tyro can read it. It is made in various sizes, and sold at various prices, and deserves to come into extensive use.

from *Nature*, 13, 135; October 16, 1875

spread distribution within the disturbed forest.

A recently published pollen diagram from eastern Finland by Hicks (*Commentat. Biol.*, 80, 1; 1975) is of considerable interest in this debate. It shows an expansion of *Picea* at about 3,000 BC yet evidence of agriculture does not occur until a level dated at about 300 years ago. This is consistent with historic records of the invasion of the area by Finnish farmers during the 17th century. Previously northern Finland had been occupied by herding and hunting Lapps. There is still the possibility that some forest management, such as burning, could have been practised, however; perhaps the pollen type *Melampyrum* in Hicks' diagrams are indicative of this.

In Britain we have been spared these problems because *Picea* has never reached these islands during the present interglacial. An equally enigmatic problem, however, is posed by the marked decline in pine, *Pinus sylvestris*, since 5,000 BC and particularly in the last 4,000 years. This has affected the species in Ireland (Pilcher, *New Phytol.*, 72, 681; 1973) where Bronze Age peoples may have been involved in the clearance of pine forests. Pennington (CBA Rept. No. 11, 74; 1975) has described a similar process from the uplands of the Lake District, though there the process

began in Neolithic times. Birks (*Phil. Trans. R. Soc. Lond.*, B270, 181; 1975) has found that most of the pine stumps in the blanket peats of Scotland pre-date 4,000 B.P., but here death was frequently found to be due to rising water tables, possibly a consequence of minor climatic fluctuations. In none of the cases examined was it possible to blame man for the death of the trees. In the southern Pennines, on the other hand, Tallis (*Nature*, 256, 482; 1975) regards the death of at least some of the trees preserved as stumps in the peat to be the result of human forest clearance.

In the case of both spruce and pine it is difficult to unravel the interacting influences of climatic changes and human clearance. It is evident, however, that man's involvement with these needle-bearing conifers began long before the yule log became a component of his culture. □

Does the Earth's field reverse?

from Peter J. Smith

THE magnetic properties of natural lodestone have been familiar for a long time; indeed, the ancient Greeks knew at least 2,000 years ago that lodestone would attract pieces of iron, and by the first century AD the Chinese had invented the compass in the form of a lodestone spoon rotating upon a smooth board. In recent years, however, it has become apparent that even quite common rocks may exhibit magnetic characteristics. The fact that some eruptive rocks possess a natural magnetism much larger than can be acquired in the present geomagnetic field was reported by Arnim (*Ann. Physik*, 5, 384; 1800) and confirmed by Locke (*Trans. Amer. Phil. Soc.*, 9, 283; 1846). Fournet (*Ann. Sci. Phys. Nat.*, 11, 134; 1848) has suggested that such a phenomenon is possible because of the presence in the rocks of magnetite. Unfortunately, this idea does little to explain how rocks can acquire an ordered magnetism; and the role, if any, played by the Earth's magnetic field remains obscure.

The mystery has now deepened with the publication of an article by Broun (*Geol. Rep. R. Soc. Lond. Trans. Sect.*, 24; 1860), who concludes that the direction of magnetism in some rocks may be quite different from that of the geomagnetic field. The rocks in question come from Moocoonoomalley, a granite hill which rises some 800 feet above sea level near Trevandrum in India. Broun has taken several samples from the hill, having previously marked their positions with respect to

the geographic coordinates, and has measured their effect on a freely suspended magnet at various orientations.

All the samples studied have determined magnetic axes with north and south poles at opposing faces. And the magnetic axes are always aligned roughly geographic north-south, suggesting that the magnetism owes more to the effect of the Earth's magnetic field than to any natural divisions or 'lines of crystallisation' in the rocks themselves. But whereas in most of the samples the north magnetic poles were directed towards the true north as expected, in some the north poles pointed south. Broun concludes that in the latter cases "we cannot suppose that the magnetism of the small magnets has been due to the inducing action of the earth in their present position or since the rock mass became solid". There is a clear implication here that these rocks acquired a permanent magnetism before, or as, they cooled from their molten state, presumably under the influence of the Earth's magnetic field.

But could this possibly mean that at some time in the past the Earth's magnetic field pointed in the direction opposite to the present direction, giving rise somehow to rocks with 'reversed' magnetism? Broun is not so bold as to suggest any such thing him-



de Castro: first discoverer of reversed magnetism?

self; but his work inevitably brings to mind two previous articles which went largely unnoticed at the time of their publication. Some years ago, Humboldt (*J. Phys. Chim. et Hist. Nat.*, 2, 314; 1794) reported that when he brought his compass near a large mass of very pure serpentine atop a granite hill in the Fichtel Mountains of Upper Franconia, the needle completely reversed its direction. His conclusion was that the magnetic axes in the serpentine are parallel to the Earth's field but 'reversed' in polarity. Unfortunately, measurements carried out on the same rock by others in 1816 indicated that the polarity reversal was less complete over the whole mass of serpentine than Humboldt had thought, raising some doubt as to the true picture. Seen in the light of the more detailed study of reversed magnetism by Broun, however, Humboldt's discovery must be taken more seriously than hitherto.

An even older example of a compass needle reversing its direction was presented by Castro (*Logbooks of João de Castro*, Portugal, 1538-1539). During an expedition to the coast of India, Castro landed on the Island of Chaul and, placing his compass on a boulder in order to measure the bearings of the island, found that the north end of the

needle pointed south. Although he made sure that the rock was not lodestone, Castro failed to record whether or not the boulder was in its original position of formation. The direction of the magnetism in it therefore remains unknown. But magnetised the boulder certainly was; and so Castro must be credited at least with the discovery that rocks other than lodestone can be magnetic.

In summary, then, Castro discovered magnetic rocks, Humboldt discovered reversely magnetised rocks and Broun has demonstrated scientifically that reversed magnetism is genuine. Taken together these are exciting results which pose some fascinating problems for the future. In particular, is a reversal of the Earth's field polarity really conceivable? If, as some still believe, the Earth is less than 6,000 years old, such a reversal might pose several problems of timing, for it is hardly to be imagined that so radical a change in so fundamental a phenomenon as the Earth's magnetism would be rapid. On the other hand, if, as Phillips (*Life on Earth: Its Origin and Succession*, Macmillan, London, 1860) suggested earlier this year, the Earth is 96 million years old, this difficulty would recede. □

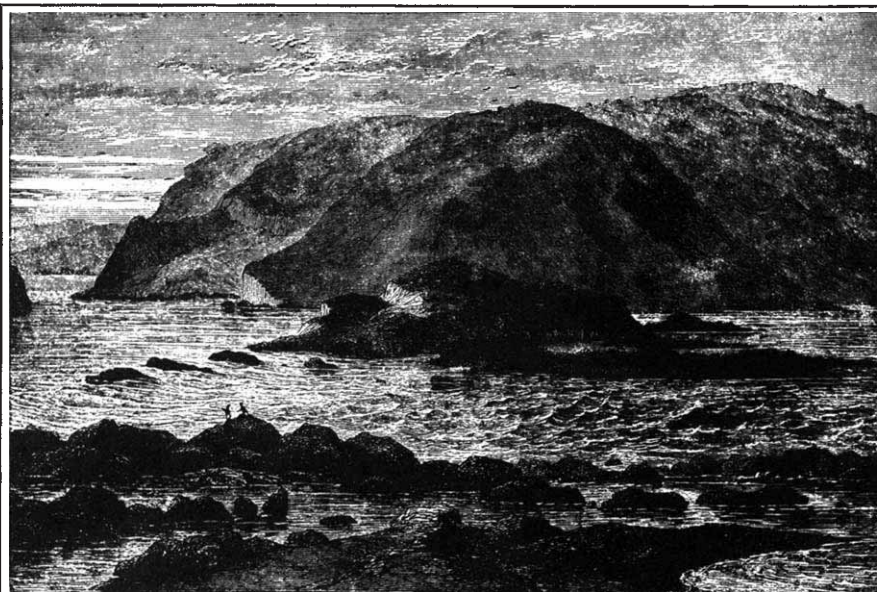
Second messengers in synaptic transmission

from a Correspondent

A satellite symposium to the Fifth International Meeting of the International Society of Neurochemistry entitled New First and Second Messengers in Nervous Tissues, was held at Brescia, Italy, on August 28-30, under the auspices of the Department of Pharmacology of the Brescia Medical School. It was organised by the Chairman, Dr Marco Trabucchi.

Cyclic nucleotides that function as second messengers in synaptic transmission not only mediate the first messenger-elicited changes in ionic membrane mechanisms but also participate in the regulation of the expression of the metabolic code. They can either enhance template activity or increase RNA polymerase II activity. This important progress in our understanding of how metabolic responses are regulated trans-synaptically in post-synaptic cells was reported during the symposium.

D. M. Chuang *et al.* (National Institute for Mental Health, Saint Elizabeth's Hospital, Washington, DC) reported that in rat adrenal medulla during a trans-synaptic sustained stimulation of postsynaptic nicotinic receptors or by the injection of specific receptor agonists, the 3',5'-cyclic adenosine monophosphate (cyclic AMP) content increases by about 10-fold. The onset of this increase is coupled with stimulus application, but its duration is regulated independently. They have shown that a complex self-regulatory mechanism involving protein kinase, an activator of cyclic nucleotide phosphodiesterase and a high K_m cyclic nucleotide phosphodiesterase regulates the extent and duration of the cyclic AMP accumulation elicited in chromaffin cells by sustained trans-synaptic stimulation of adenylate cyclase. A low molecular weight protein, stored in the membrane of chromaffin cells, can be released *in vitro* from membrane preparations of adrenal medulla following phosphorylation of membrane proteins by a cyclic AMP-dependent protein kinase. As described by Cheung (*J. biol. Chem.*, 246, 2859; 1971), this low molecular weight protein activates cyclic nucleotide phosphodiesterase by lowering the K_m of the high K_m enzyme which possesses a high catalytic capacity. Hence, in chromaffin cells, by lowering the



The Yellala (rapids) of the Congo River.

Two Trips to Gorilla Land and the Cataracts of the Congo. By Richard F. Burton. Two vols. (London: Sampson Low and Co., 1876.)

The chief interest of the second volume is connected with the Congo river, up which Burton journeyed as far as the Yellala, or rapids, which he calculates to be between 116 and 117 miles from the mouth, the total fall in that distance being 390 feet, of which 195 feet occurs between the Yellala and Boma, 64 miles. From the Great Rapids to the Vivi or lowest rapids, a distance of five miles, the fall is 100 feet. Some important facts are given as to the character of the Congo

mouth and the changes which are constantly taking place, which must even yet be of value to chart constructors.

The author gives a minute and graphic description of the river and its many reaches between Boma and the rapids; the scenery on the banks is often quite Rhine-like in its character. The river itself Capt. Burton regards as one of the noblest in the world. With a valley area of 800,000 square miles, it has a yearly mean volume of 2,500,000 cubic feet per second, nearly four times that of the Mississippi, which has a very much larger drainage area.—(*Nature*, 13, 123; October 16, 1875).

K_m of the phosphodiesterase the trans-synaptically induced increase of cyclic AMP content can be terminated even in the presence of persisting trans-synaptic stimulation of adenylate cyclase. When the cyclic AMP content of chromaffin cells is enhanced for one hour or longer, the cytosol protein kinase—the receptor protein for cyclic AMP—is activated. This enzyme is formed of two subunits: a regulatory (R) and a catalytic (C) moiety; normally the inactive RC molecular configuration is the most abundant form of the enzyme. When cyclic AMP increases it binds to R and dissociates the RC complex. As a result, the protein kinase activity is enhanced because when C is dissociated from R, it can express its catalytic activity at a maximum. Following persistent trans-synaptic activation of chromaffin cells most of the protein kinase molecules in these cells are dissociated and the C form prevails. This increase of C subunits lasts for about 4 h; however, the duration in the decrease in the number of RC molecules lasts for about 15 h. A. Guidotti and collaborators (NIMH) have reported that the mechanism whereby the trans-synaptically induced increase in C subunits is terminated is not by the reassociation of C with R but by the translocation of C from cytosol to sub-cellular structures including the nucleus. Chuang and E. Costa (NIMH) have shown that coincident with this translocation there is an increase in the synthesis of nuclear poly(A)-RNA. This elevation of mRNA synthesis rate is followed by an increased synthesis of tyrosine-3-mono-oxygenase (TH), the rate limiting enzyme for catecholamine biosynthesis. Also the synthesis of other medullary protein is enhanced at this time.

D. Russell (University of Arizona) also reported that the cyclic AMP content of adrenal medulla increases following the trans-synaptic activation of nicotinic receptors. She has shown that this second messenger response triggers the activation of ornithine decarboxylase which, in turn, accelerates the synthesis of ribosomal RNA.

R. A. Jungman and E. G. Kramias (Northwestern University Medical School, Chicago) have suggested that during hormonal regulation the RNA polymerase II activity can be modified through a phosphorylation of the enzyme by the action of a nuclear cyclic AMP-independent protein kinase.

I. Hanbauer (National Heart and Lung Institute, Bethesda) reported that when rats are maintained for about 1 h in an environment with low O_2 tension, the TH activity of the carotid body increases after an interval of about 24 h following the stimulus. This

increase in TH activity is also preceded by an activation of the cyclic AMP system. She reported that this chemical stimulus fails to change the TH in adrenal medulla or in superior cervical ganglia; moreover, she presented evidence that this TH activation does not require the participation of adrenergic or cholinergic mechanisms.

During this symposium, E. Giacobini (University of Connecticut, Storrs) and M. Otsuka (University of Tokyo) and others presented substantial experiments supporting the view that first messengers may include not only amino acids and their metabolites, but also polypeptides and adenosine triphosphate. The biochemical and functional implications of the participation in synaptic transmission of several molecular entities of transmitters were debated. It was also discussed whether the presence of such a pluralistic system of first messengers may require an updating of the Dale postulate that each neurone contains one and only one transmitter. □

Fighting erosion with soil conditioning

from A. J. Low

The third international meeting on Soil Conditioners was held at the University of Ghent in Belgium on September 8–12. The proceedings of the symposium (edited by M. De Boodt) will be published by the Faculty of Agricultural Sciences of the University of Ghent.

SOIL is a three phase system—a porous heterogeneous mixture of solids (minerals and organic matter), liquid (water containing many solutes) and gases. The structure of a soil is determined to a large extent by the soil organic matter which holds together the mineral skeleton. Soil structure is developed by the action of the root systems of permanent covering vegetation, such as that of grasslands. But when grassland is cultivated, the organic matter which has formed is quickly broken down and there is a corresponding structural collapse. The natural soil 'cements' include polysaccharides and polyuronides, and soil conditioners are synthetic replacements for these.

The first soil conditioners were probably vinyl acetate—maleic acid and hydrolysed polyacrylonitrile. Mixed with soil at a suitable moisture content they can bring about a much more stable structure which can last for ten years or so. Marked increases in crop yield have occurred when structure has been the limiting yield factor, but soil

conditioners have not generally been adopted on a large scale because of their high cost.

What may be an important development is the application of soil conditioners in arid regions such as North Africa where to prevent the spread of the desert northwards an attempt is being made to establish a belt of trees (the Barrage Vert). The desert soil is largely devoid of organic matter, unaggregated and subject to wind erosion. It can be stabilised by building aggregates with soil conditioners and thus providing the conditions for trees and other plants to become established after planting which in turn helps to reduce wind erosion.

Suwardjo and Lenvain (Bogor Soil Science Institute, Indonesia) described how re-afforestation was being carried out in the humid tropics on strongly eroded areas where the soil structure was weak, by stabilising the soil in and around the planting holes of young trees. Once the trees are established the surrounding area tends to become revegetated.

W. Flaig (West Germany) related work on the biochemistry of soil organic matter to work on soil conditioning. He concerned himself especially with high molecular weight naturally occurring organic polymers and briefly discussed the possibility of converting lignosulphonates and other waste products into soil conditioners. The soil conditioning effects of lignin derivatives, sewage sludge and other waste products were also reported by Vasiljev (USSR), M. B. Kirkham (USA) and Kottnerus (Netherlands).

Fundamental studies of soil conditioners as materials for changing soil physical properties were described by De Bisschop (Ghent University) and Koenigs (Wageningen University) particularly liquid distribution in porous media.

Soil conditioners have been used to stabilise the backfill of pipe drains (Dierickx and Gabriels, Ghent University), in rice culture (Keersebilck and Muljadi, Bogor) and in stabilising sugar beet seed beds where soils are liable to cap.

The symposium concluded with an analysis of the papers and the conclusions to be drawn by Dudal (FAO) who suggested that soil conditioning might now be ready for application on a larger scale than hitherto. In contrast to the first symposium more than 50% of the papers in the third dealt with the use of waste products as soil conditioners. This was undoubtedly due to economics. The synthetic polymers used as soil conditioners in the 1950s were even then very expensive and continue to be so.

The fourth symposium is to be held in Australia on August 16–23, 1976.

Christmas quiz

1. (a) Did Mendel have to cheat to discover the fundamental laws of genetics?
(b) Why were his results not recognised for forty years?
2. Norbert Wiener, Julian Schwinger, James Watson and Murray Gell-Mann were all child prodigies. But which was the most prodigious?
3. James Sumner and John Cornforth both obtained Nobel prizes for chemistry, albeit 29 years apart. What else did they have in common?
4. A question for women's year: The female is often larger as well as more deadly than the male. In which species is the imbalance greatest?
5. In which film, made in 1944, was there a sub-plot of German agents trying to smuggle uranium out of America?
6. In which book:
 - (a) was there a submarine powered by the same force as powers the Sun (written 1870);
 - (b) did Japan launch a surprise aerial attack on the west coast of America (written 1908);
 - (c) did scientists develop strains for biological warfare (and subsequently use them on China, the emergent world power) (written 1914);
 - (d) did a group of 'conservationists' fight the profligate squandering of natural resources by big business (written 1952)?
7. Whose correspondence was found recently in the Old and New Worlds, and what was the subject discussed?
8. For they have sown the wind, and they shall reap the reward. When was this biblical warning renewed recently, and what human activity was said to be the cause?
9. Which animal with ecclesiastical connections may aid investigations of human demyelinating disease?
10. What was the first anorectic drug used in the treatment of obesity?
11. What effect is season of birth supposed to have on the incidence of schizophrenia?
12. When was the first purely scientific journal founded?
13. Who's got arms coming out of his head?
14. Who invented the linear motor and ran away from the Royal Institution?
15. Who began by counting from 1 to 10 in binary form, added on the atomic numbers of hydrogen, carbon, nitrogen, oxygen and phosphorus, then twice 4,000, and drew a cuboid biped 14 wavelengths high with a helix on its head? And who turned it upside down?
16. In spite of the demise of flower children, strings of beads are still in favour with one group. Who are they?
17. Who said:
 - (a) Science is the cemetery of dead ideas;
 - (b) Science is the topography of ignorance;
 - (c) Science is nothing but perception;
 - (d) Science is the great antidote to the poison of enthusiasm and superstition?

See page 556 for answers.

Miscellaneous intelligence

● "Contrary to the trends in all other countries in the European Economic Community the rate of movement into human consumption in Great Britain increased during the three previous seasons (and shows a provisional offtake for 1974/75 of 222 lb per head per annum)." So says the Potato Marketing Board in its latest annual report, meaning that although the British people had been eating more potatoes during the past few years, they now seem to be eating fewer. Does this indicate that they might be in a spud-deficient situation?

● Oh for the elegant style of yesteryear. Lecturing at Gresham College, London on October 26, 1681, John Flamsteed, first Astronomer Royal, warned: "Hee that has not a Competent understanding in Geometrie, a thorough knowledge of plaine & sphericall Trigonometrie, with the Severall Theories of the planets motions, hee that with these is not well acquainted with the Sphere it selfe, & its severall projections & knowes not readily the Constellations, ought not to pretend to the businesse in which all these are required . . ." For further enjoiment, even if you knowe not one end of a telescope from ye other, see ye full texte in *The Gresham Lectures of John Flamsteed*, edited by E. G. Forbes (Mansell, London, 1975).

● A recent joint meeting of the Society of Chemical Industry and the Society for Occupational Medicine heard the momentous news that dialogue between the professions of chemistry and medicine was difficult in that each tended to use its exclusive jargon.

● Elsevier has published yet another journal. *Mass Emergencies* is intended for everybody concerned with problems of emergencies and disasters. It is to be hoped that it soon tells them what to do about an avalanche of new journals.

● A new book from Oxford University Press has a chapter—Education: how to make the best use of your brain—which might have been the answer to many a prayer. Sadly for aspiring geniuses, it describes no tricks for improving the power of mind over matter. Keeping to the realms of the possible, it deals largely with the acquisition of human skills. (*Explaining the Brain* is by Ritchie Russell with A. J. Dewar.)

● Television talks for small planters have been delivered by the staff of the Mauritius Sugar Industry Research Institute, according to the institute's latest annual report. Bad luck tall planters.

Results of competition No. 3

Examples of momentous discoveries happened upon by chance proved disappointing, and no first prize has been awarded. Consolation prizes go to the authors of the stories printed below, although one contains a historical inaccuracy.

"And now, allow me to demonstrate my new, cheap, thin-wire, glass enclosed heater", said Edison, slowly closing the switch. The ammeter flicked over and the lab. was lit up by a flickering glow. "Of course this is just to show the principle", he added heartily. "The production model will have opaque glass walls and a heat efficiency better than 80%".

D. R. REED

It is said that Arne Tiselius developed cellulose column chromatography from an ancient practice of Swedish peasants. While on holiday in the forests of western Sweden he came across an old woodcutter who, to prepare lunch, broke the ends off a long loaf of bread, stood the truncated part upright in a jar, and poured into the top of it a crude distillate of pulped leaves; the woodcutter ate his crusts after dipping them into the liquid which eventually filtered into the jar, and gave his dog the rest of the loaf. Realising that he had witnessed an ingenious method of separating ethyl and methyl alcohols, Tiselius returned to his laboratory pondering on how to apply it to the purification of proteins.

D. ROSEN

It is reported that Gregor Mendel once made a courtesy visit to a neighbouring nunnery. A keen gardener himself, he was much impressed by the magnificent sweet pea beds leading to the main entrance. The gardener, a tall, handsome, young red-haired man (rare in those parts of Moravia), accepted Mendel's compliment as nothing less than his due.

The abbess showed Mendel around, and was particularly enthusiastic about the success of their orphanage and school. Mendel, however, was more taken with the surprisingly large number of tall, pretty, red-haired children attending. "Strange", he thought to himself . . .

F. H. COOPER (after BOCCACCIO)

"Get back here and put some clothes on!" shouted Mrs Archimedes.

By that time, however, her husband was halfway to the palace, and Mrs Archimedes was left standing ankle-deep in spilled bathwater. How could she possibly clean that mess? Suddenly she noticed that the hem of her gown was soaking up some of the spillover. With a shout of "Eureka!", she quickly tore up some old clothes, tied them to a stick, and invented the mop.

S. GILBERT

Competition No. 4

One of the most popular sections of *Nature* is 100 Years Ago. Readers are invited to submit 100 words culled from the pages of *Nature* 100 years hence. Closing date: February 1, 1976.

Ghost lecturers

When Professor Richard Eakin's lectures declined in popularity and audiences dwindled, he hit upon a topping wheeze. To revitalise his course in elementary biology he would bring in guest lecturers of exceeding eminence—Mendel, Darwin, Pasteur and their ilk. Being a thorough worker, and having at his disposal the considerable resources of the University of California, Berkeley, he has been able to transmogrify himself into various scientists who long since passed into the annals of history.

Thus when it is time to discourse on blood, an Elizabethan gentleman steps forth, carrying a Latin treatise, a heart and a pitcher of tomato juice. He is William Harvey and the props serve to demonstrate his work on the circulation of the blood. Later during the course of thirty lectures (most of which are delivered by Professor Eakin playing himself) Harvey is followed by William Beaumont, the gruff nineteenth-century American army surgeon who pioneered the investigation of gastric juice and digestion. He holds forth with some relish about his investigations with his patient, and later guinea pig, Alexis St Martin, a fur trapper whose stomach was shot open and remained open through the wall of his abdomen even after his recovery.

Embryology is introduced by a frock coated Herr Professor Dr Hans Spemann, the only character in Eakin's repertoire whom he knew. Genetics is brought into the course by a jolly, cigar smoking Brother Gregor Mendel, complete with plastic sweet peas (always fresh and available). To add authenticity to his portrayal of Louis Pasteur, Eakin has taken lessons in the art of affecting a French accent. He is apparently acquiring the Queen's English to improve his interpretations of Harvey and Darwin.

After their local success, the guest lectures have now been published in a book with the alluring title of *Great Scientists Speak Again* (by Richard M. Eakin, University of California Press, Berkeley, Los Angeles, London, 1975, £4.50). Other biologists eager to give the best attended lectures are invited to get out their grease paint, wigs and pitchers of tomato juice.

M.L.

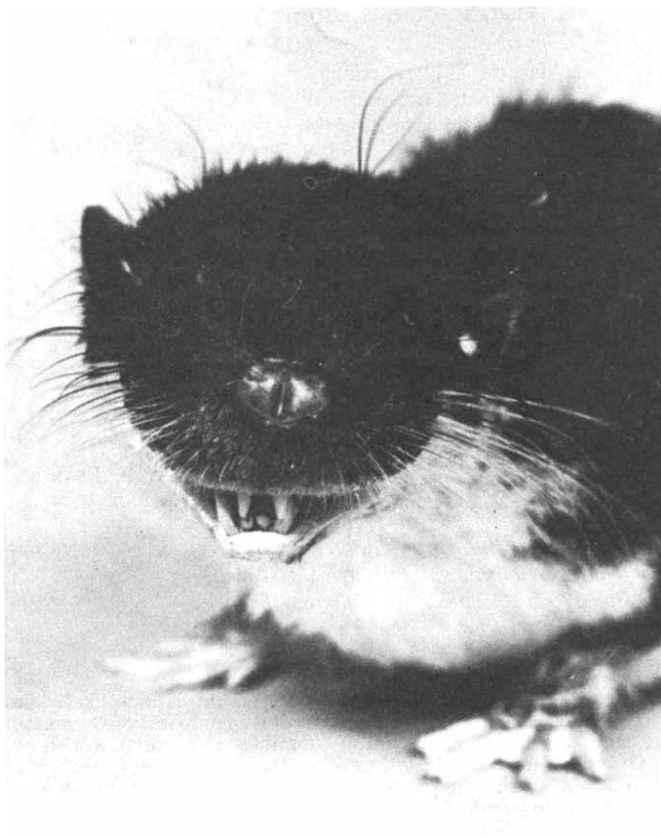


Photo by A. Friday

This vicious beast was caught by natives in deepest Zaïre on behalf of two British zoologists who risked disease and discomfort to secure a sample of its blood. In spite of its fearsome appearance, this West African otter shrew, here slightly larger than life, is stuffed and safely at rest in the Museum of Zoology at Cambridge (see *Nature*, **258**, 107; 1975).

Ode to an eddy

The mighty stream Agulhas
Wends its wand'ring way
Down the coast of Afrik
And on past Durban Bay¹.

'Twas there we found The Eddy²,
Majestic and serene,
Cyclonically rotating:
The best we've ever seen.
Its heart was cool yet shallow—
Its motion slow and steady—
Its structure well-defined—
Ah, yes, that was an eddy!

We know not of its birthplace,
Nor when it first saw day,
But Gründlingh has a theory:
They're born off Richards Bay³.
The coastline at St Lucia
Deflects the Mighty Flow,
And southwards in its wake
The eddies form and grow.

Yet that's not all the answer,
For other data shows
The inshore flow is governed
By the weather-bringing "lows"^{4,5}.
These cells of falling pressure
Arrive from west-sou-west
And bring the dreaded "busters"⁶
Which the mariners detest.

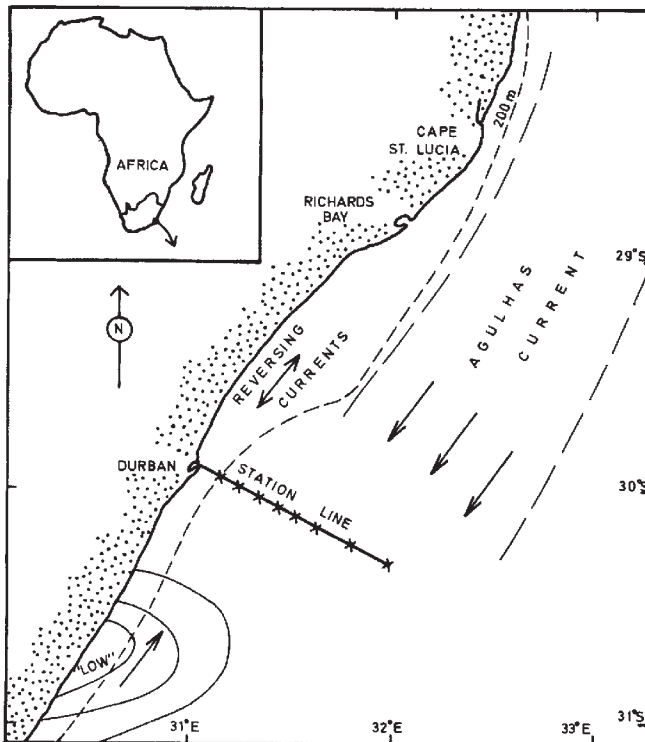


Fig. 1 Location chart, illustrating diagrammatically the general flow of the Agulhas Current, the reversing current on the continental shelf, the migration of a typical cyclonic cell from the south and the line of eight oceanographic stations. This line was occupied daily for the 5 d period February 19–23, 1973.

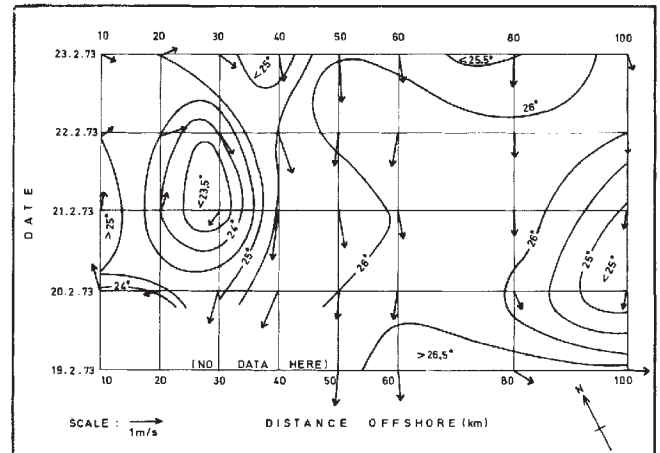


Fig. 2 The eddy is shown by the sea-surface temperature contours (4°C intervals) and the current vectors measured at 10 m depth during the 5 d of the cruise.

And years of observation
Along the eastern coast
Suggest that eddies form
When pressure's down the most.
It therefore seems quite likely
That instead of from the north
It's really from the south
That the eddies issue forth.

Alas, our single section
(Repeated occupation)
Did not show the direction
Of eddy propagation.
Is it to north or south
The eddies set their face?
Or maybe they are born
and die in the same place?⁷

We're planning now a programme
To show them in their glory.
We'll seek them—find them—track them—
Until we know their story⁸.

A. F. PEARCE

National Research Institute for Oceanology,
Council for Scientific and Industrial Research,
PO Box 17001, Congella 4013, South Africa

¹ See the location chart in Fig. 1.

² Fig. 2. A more detailed publication on this and other eddies associated with the Agulhas Current is in preparation (at present they are described in various unpublished reports).

³ Gründlingh, M. L., *Deep-Sea Res.*, **21**, 47–55 (1974).

⁴ Anderson, F. P., Sharp, S. O., and Oliff, W. D., *Symp. Oceanography in South Africa*, Durban, 1970, paper H2, page 22.

⁵ Pearce, A. F., *South African National Oceanographic Symp.*, Cape Town, 1973, page 28.

⁶ The "lows" move up the coast from the south at intervals of a few days, and bring in their wake south-westerly winds; when these are of gale intensity, they are termed "busters".

⁷ It is of course possible that standing eddies may be generated by the sudden change in the 200 m isobath north of Durban—there is evidence of semi-permanent patches of cooler water off Durban, shown by airborne radiation thermometer surveys.

⁸ Eddies of a similar (?) nature associated with the Gulf Stream system have been described by Lee, T. N., dissertation, Florida State Univ. (1972).

review article

Control of growth of mammalian cells in cell culture

Robert W. Holley*

Recent studies of the control of growth of mammalian cells are considered and it is concluded that the factors that control growth are probably polypeptide hormones, or hormone-like materials, as well as a variety of low molecular weight nutrients. An understanding of the control of growth of mammalian cells will probably be found in the complex interactions of cells with these common types of materials.

ORDERED, controlled growth is essential for the development and maintenance of a normal animal. The mechanisms that control growth are selective for different types of cells within an animal and are responsive to changes in the environment of cells, as, for example, after wounding.

Some aspects of growth control are observed in cell culture. In general, normal cells in culture show more restricted and ordered growth than their transformed or malignant counterparts. The extent to which growth controls are lost in cell culture parallels the tumorigenicity of the cells *in vivo* in some instances^{1,2}, suggesting that the growth controls seen in culture have relevance *in vivo*.

Two questions are of particular interest. What factors control the growth of normal cells? Why do the same factors not control the growth of transformed or malignant cells?

"Density dependent regulation" of growth

Normal, untransformed cells show "density dependent regulation", that is, they tend to grow to a certain "saturation" density and then stop growing. The resulting quiescent cells can remain both healthy and quiescent for some time. Highly transformed cells, in contrast, have lost "density dependent regulation" and they grow continuously until they exhaust the supply of low molecular weight nutrients in the medium; the cells do not become quiescent, and they must be transferred to fresh medium or they die. To understand the difference between normal and transformed cells, it would be helpful to know what factors control the growth of normal cells during "density dependent regulation".

Initially, "density dependent regulation" was ascribed to "contact inhibition"³, in analogy with contact inhibition of movement of cells⁴. It was postulated that contacts between crowded cells repress further cell proliferation. Although the term "contact inhibited" is still used in the literature to describe quiescent cells, many lines of evidence indicate that quiescence is not caused by contacts between cells. Instead, arrest of growth of mammalian cells during "density dependent regulation" appears to be the result of

limitation of any one of a variety of materials in the medium surrounding the cells.

Factors in the control of growth

In "density dependent regulation", cells grow to their "saturation density" and become quiescent. The "saturation density" varies with the type of cell and the culture conditions. Though few situations have been studied in detail, it is clear that many different factors can control growth in this way. The factors encountered in routine cell culture fall in to two broad classes. They may be either macromolecular factors in serum^{2,5,6}, or low molecular weight nutrients in serum⁷ or the synthetic medium⁸⁻¹¹.

"Density dependent regulation" that involves macromolecular serum factors is of particular interest, and, for reasons that will be discussed below, emphasis in this review will be placed on serum factors. The growth control that is observed is, however, at least superficially, the same when growth is controlled by low molecular weight nutrients. The cells grow to a predictable cell density, and after growth stops the quiescent cells are found primarily in the G₁ or G₀ phase of the cell cycle^{8,11}. Growth reinitiates if the limiting factor is added to the culture^{8,11}. There is evidence that the cells are arrested at the same "restriction point" whether the limiting factor is a low molecular weight nutrient or a macromolecular serum factor¹². One difference that is observed when cultures are limited by low molecular weight nutrients is that it is more difficult to maintain the cells both quiescent and healthy, presumably because the nutrients have essential metabolic roles in addition to their growth regulatory roles.

The conclusion that the growth of mammalian cells can be controlled by many different factors is strengthened by experiments which deliberately vary the culture conditions with a single type of cell. For example, the growth of 3T3 cells, a mouse embryo cell line, is normally controlled by macromolecules in serum^{2,5,13}. The "saturation density" is directly proportional to the initial serum concentration in the medium^{5,13}. If the experimental conditions are varied, however, the growth of these cells can be controlled, at least to some extent, by any one of the following pure materials: fibroblast growth factor^{14,15}, epidermal growth factor¹⁶, insulin^{15,17}, hydrocortisone¹⁸, prostaglandin F_{2α}¹⁹,

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cyclic nucleotides²⁰, calcium ions²¹, amino acids¹¹, phosphate ions¹¹, glucose¹¹, and proteolytic enzymes²².

The list of growth-controlling factors becomes even longer and more varied if other mammalian cell types are included. The list includes, besides the general types of materials listed above, peptides^{23,24}, antigens²⁵, lectins²⁵, and lipopolysaccharides^{25,26}. The length and variety of this list suggest that the mechanism that controls mammalian cell growth is responsive to some sort of complex interaction among many factors.

Polypeptide factors probably control growth *in vivo*

Of the list of pure materials that control the growth of 3T3 cells, the two polypeptides, the fibroblast growth factor and insulin, are active at extremely low concentrations. The two act synergistically and their action is potentiated by a glucocorticoid¹⁵, reminiscent of the action of prolactin, insulin, and a glucocorticoid, on the growth of mammary gland cells²⁷⁻²⁹. Because they are active at such low concentrations, and because they could interact specifically with cell surface receptors on selected cells, it seems likely that the polypeptide factors represent the important, natural macromolecular factors that control growth of mammalian cells *in vivo*. Consistent with this view is the extensive list of similar polypeptide factors. The list includes nerve growth factor³⁰, epidermal growth factor³¹, colony stimulating factor³²⁻³⁴, the somatomedins^{35,36} and non-suppressible insulin-like activities³⁷, rat fibroblast growth factors S1 and S2³⁸, mesenchymal factor³⁹ and ovarian growth factor⁴⁰, as well as the many polypeptide hormones that are known to control growth of tissues *in vivo*. Similar factors, present in blood platelets, may be of significance in wound healing^{41,42}. Certain of the polypeptide factors seem to be released into the culture medium by cell lines^{32,37}. Numerous growth controlling factors probably remain to be identified.

Roles of other factors

If polypeptide hormones and similar growth-controlling serum factors have the key role in controlling mammalian cell growth, then what roles are played by the many other types of materials, listed earlier, that control cell growth in culture?

Many of these materials probably mimic hormone action. For example, proteolytic enzymes and other hydrolytic enzymes may, when they stimulate growth, act on hormone receptor sites⁴³, or they may alter the cell surface in ways that potentiate the action of polypeptide hormones. Small peptides that are active as growth factors for some cell lines may also mimic hormone action. Exogenous cyclic nucleotides may affect growth by providing one of the products of polypeptide hormone action⁴⁴. Antigens, lectins and lipopolysaccharides interact with lymphocyte cell membranes and, again, may activate cell proliferation by mechanisms that are similar to the actions of polypeptide hormones on the membranes of other cells.

Common low molecular weight nutrients may or may not act by the same general mechanism. Low molecular weight nutrients seem to be able to regulate growth of most cells, suggesting that regulatory mechanisms involving such nutrients have important roles. Since similar mechanisms are observed in microorganisms, for example, in the "stringent" amino acid control of bacteria⁴⁵, these growth control mechanisms may be very primitive in origin. They may be important in animals in starvation conditions, and although they may be completely unrelated to the action of the polypeptide hormones and growth factors, an intriguing possibility is that the hormones and

growth factors act by invoking the same intracellular growth control mechanisms affected by nutrients⁴⁶.

In addition to all the factors that stimulate growth, other polypeptide factors, and possibly "chalones"⁴⁷, may act to inhibit growth of cells. An example of the inhibitory action of a polypeptide hormone is the suppression of growth of adrenal tumour cells by ACTH⁴⁸.

Roles of polypeptide factors in "density dependent regulation"

Assuming that the polypeptide factors are the important growth-controlling factors, what is their role in "density dependent regulation" of growth? Two kinds of situation can be studied experimentally. Cells can be studied at different densities in different culture dishes, or, using special manipulations, they can be studied at different densities in the same culture dish.

With 3T3 cells, growth can be arrested at any cell density by choosing the appropriate serum concentration¹³. Whatever the cell density, initiation of DNA synthesis takes place in the quiescent cells if the concentration of serum or the pure polypeptide factors is raised. The higher the original quiescent cell density, the higher the serum or polypeptide factor concentration must be raised to initiate DNA synthesis.

The most dramatic demonstration of "density dependent regulation" involves making a "wound" in a quiescent, confluent cell layer by removing a narrow strip of cells^{2,49,50}. Cells migrate in from the edge of the "wound", initiate DNA synthesis and divide, and cell proliferation eventually fills in the "wound"^{2,49}. Proliferation of sparse cells within the "wound" takes place in the same medium in which crowded cells, in the confluent layer, remain quiescent.

In the "wound healing" experiment with 3T3 cells it can be shown that serum factors are essential. Without serum in the medium, cells at the edge of the "wound" do not migrate into the open area and the cells do not initiate DNA synthesis^{50,51}. A fraction has been obtained from serum that promotes migration of 3T3 cells into the "wound" in the absence of serum, but DNA synthesis is not initiated in these sparse cells unless the macromolecular factors that control growth are also added⁵¹. The same factors initiate DNA synthesis in the sparse cells and in the crowded cells, but a lower concentration of factors is effective in sparse cells⁵¹.

These results in the "wound healing" experiments with 3T3 cells suggest that the same macromolecular factors that control normal growth are the important factors in "density dependent regulation".

The major inconsistency in the literature is the report that certain epithelial cells have a negligible "wound serum requirement"⁵⁰, that is that these cells initiate DNA synthesis in the "wound healing" experiment in the complete absence of serum. This suggests the possibility that epithelial cells show some other kind of growth control. This question has been reinvestigated using BSC-1 cells, an established line of African green monkey kidney cells. It has been found that the growth of BSC-1 cells, in either sparse or crowded culture, or in a "wound", is dependent on a low level of macromolecular serum factors (unpublished work). The earlier report that initiation of DNA synthesis takes place in BSC-1 cells at the edge of a "wound" in medium without serum seems to be due to difficulties in washing away all the serum factors when the cells have been grown to confluence in 10% serum.

It seems likely, therefore, that "density dependent regulation" is due to a quantitative increase in the requirements for macromolecular growth factors as cell density increases.

Mechanism of "density dependent regulation"

It is not clear why crowded normal cells need a higher concentration of the macromolecular factors for growth. It is known that the polypeptide growth factors are destroyed by the cells⁵. Increasing the cell density probably increases the rate of destruction, and there is evidence that destruction of the factors creates a diffusion boundary layer⁵². In addition it seems likely that cells become less responsive to a given concentration of the growth factors as their surface area and movement decrease, perhaps because of a decrease in the number, availability or mobility of surface receptors for the factors. There is much evidence that the surface area of the cells⁵³⁻⁵⁵ is important in "density dependent regulation".

Mechanisms of action of factors that control growth

Although there have been many studies of the initiation and arrest of cell growth in culture, little is known about the internal mechanisms that control cell growth. It is known that a complex set of changes follows the stimulation of growth of quiescent cells by hormones or serum, the "pleiotypic response" described by Tomkins *et al.*⁵⁶. There are changes in transport of nutrients including ions^{58,59-58}, changes in intracellular concentrations of cyclic nucleotides⁵⁹⁻⁶¹, changes in RNA and protein synthesis^{2,59}, and changes in microtubular assembly⁶². After 10-15 h, the initiation of DNA synthesis takes place. Because of the length of time involved, it seems likely that an extensive programme of events is required between the addition of the growth stimulus and the initiation of DNA synthesis. Since many new proteins are probably synthesised during this period, extensive changes in RNA and protein synthesis would be anticipated. It is not known whether the various facets of the "pleiotypic response" are under coordinated control⁵⁸, or whether all the observed changes are required to sustain the chain of events that leads to DNA synthesis. For practical reasons, attention has been focused on the very early events.

The earliest changes that have been detected following growth stimulation are changes in intracellular concentrations of cyclic nucleotides⁵⁹⁻⁶¹ and changes in membrane permeability and transport of nutrients^{58,59-58}. These very early changes are, however, characteristic of polypeptide hormone action in general⁶³. That the changes occur supports the conclusion that hormone action is the natural mechanism of growth control, but, if one accepts this conclusion, observation of the changes tells little more.

It has been proposed that cyclic nucleotides play the crucial role in growth control, with a decrease in the intracellular concentration of cyclic AMP^{59,64} or an increase in the ratio of cyclic GMP to cyclic AMP^{60,61} causing the initiation of growth. There seem, however, to be instances in which the initiation of growth is accompanied by changes in intracellular cyclic nucleotide concentrations other than those expected⁶⁵⁻⁶⁷. Also, a mutant cell has been obtained that is deficient in adenylate cyclase⁶⁸. Since calcium ions seem to be intimately involved with cyclic nucleotides, it is possible that calcium ions are critical in what is observed⁶⁹. There is also the possibility that the membrane permeability changes, caused by polypeptide hormone⁶³ or serum factor action, are themselves the crucial event in growth stimulation. These permeability changes may activate the same intracellular control mechanisms mentioned earlier that operate with low molecular weight nutrients⁴⁶.

Whatever hypothesis one favours, it seems likely that an understanding of the mechanisms of action of the factors that control mammalian cell growth will involve an under-

standing of the mechanisms of action of the polypeptide hormones.

Loss of growth controls in transformed cells

Transformed cells tend to grow to very high cell densities in cell culture. Under identical culture conditions, the growth of the corresponding normal or untransformed cells is limited by the supply of macromolecular serum factors in the medium. This suggests that transformed cells have a decreased requirement for the macromolecular serum factors and this decrease has been found. In some instances the transformed cells seem to have lost the requirements for the normal growth-controlling factors completely^{13,70,71}. In other instances the transformed cells retain the requirements but at a quantitatively lower level than normal cells (ref. 1 and unpublished work).

The causes of the loss or quantitative decrease of the requirements for macromolecular growth-controlling factors are poorly understood. It is quite possible that the causes differ with different transformed cells. In one instance, studies of a transformed cell that retains a requirement for the normal serum factor show that a decrease in serum requirement to one-tenth the original value is accounted for by the combination of a decrease in the concentration of serum required to initiate DNA synthesis and a decrease in the rate of destruction of the serum factors by the cells (unpublished work).

Either of two basically different alterations might underlie the changes that lead to the lower requirement for serum factors. One possibility is that transformation alters internal regulatory mechanisms, leading to the direct loss of control of DNA synthesis. Alternatively, transformation may lead to a product that affects the cell membrane, and membrane properties may be altered in such a way as to modify the requirements for hormone interaction with the membrane.

Neither possibility can be excluded by present evidence. Some highly transformed cells seem to have lost essentially all growth controls, consistent with direct loss of control of DNA synthesis. The growth of many transformed, tumorigenic cells can, however, be arrested easily in G₁ or G₀, and some transformed, tumorigenic cells have what seem to be modified hormone-cell surface growth controls. This seems more in keeping with the second possibility, that transformation affects growth control by changing the cell membrane. Membrane changes accompany transformation^{72,73}, but the membrane changes may be the result rather than the cause of changes in growth behaviour.

Though it is not possible at present to distinguish between the two possible mechanisms of altered growth control, many powerful approaches are now directed at this question. Studies of the actions of the gene products of tumorigenic viruses⁷⁴ and studies of the properties of isolated vesicles of plasma membranes of normal and transformed cells⁷⁵ are examples of approaches that may distinguish between the two possibilities and may lead to an understanding of the loss of growth controls that accompanies malignant transformation.

Summary

The overall picture that emerges is that growth of normal cells is probably controlled by interactions of the cells with various polypeptide hormones or hormone-like growth factors present in the surrounding fluids. As normal cells become more and more crowded they require higher and higher concentrations of the growth factors in order to grow. This increased requirement for growth factors is probably due in part to diminished surface area of individual cells when they are packed in among other normal cells, and

in part to cellular destruction of the factors, which lowers their concentrations in fluids adjacent to crowded cells. More exotic possibilities, such as control of growth by "contact inhibition", have not received convincing support.

Transformed or malignant cells escape from normal growth controls by requiring less of the hormones or growth factors. The cells initiate growth in response to lower external concentrations of growth factors. They probably destroy the factors less rapidly, and they probably maintain more surface area exposed to the surrounding fluids.

The changes in transformed cells that lead to lowered requirements for the growth factors may be the result of direct internal loss of control of DNA synthesis or to membrane changes that modify the requirements for hormone interaction with the membrane.

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articles

Evidence for earthquake triggering stress

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Earthquake time series are constructed by making counts per unit time of earthquakes with magnitudes greater than a chosen threshold. The time series from widely separated regions show strong correlations with one another, and it is suggested that tectonic plates are subjected to a fluctuating stress field that results in these changes in seismic activity.

Two assumptions are commonly made about earthquakes: first, that their occurrence has a stationary random Poisson distribution and, second, that most earthquakes are essentially local phenomena. To question these assumptions we have compared

the time variation of seismic activity at a number of locations throughout the world.

Very large earthquakes account for most of the energy release¹ and fault slip² within a seismic zone. One would expect such earthquakes to occur very systematically if the underlying plate motion is regular. In practice, however, the timing of large earthquakes at a given location seems to be almost random³. Spatially, evidence is accumulating that large earthquakes occur in sequence⁴, around a plate margin. Migration velocities of several hundred km yr⁻¹ have been attributed to the process of stress diffusion⁵.

Earthquakes smaller than 'very large' do not contribute significantly to the motion of the plates adjacent to a seismic

zone, and they are usually considered to be local phenomena. Most, one would presume, are either aftershocks, or some local reaction to the stress changes accompanying the infrequent major events. Their statistical characteristics are found to agree well with a generalised Poisson model⁶. The constancy of this process in time is, however, rather poorly understood. This is important, for it lies at the heart of methods for the estimation of earthquake risk.

Procedure

We are concerned primarily with earthquakes of small to medium size. The data used have been obtained from several earthquake catalogues. The primary data set is the list of earthquakes published (currently) by the National Earthquake Information Service of the US Geological Survey and commonly known as the PDE (Preliminary Determination of Epicentres) list. We have used this list for the period January 1, 1964 to April 30, 1974. Supplementing this, we have the Regional Catalogue of Earthquakes published by the Japan Meteorological Agency (January, 1964 to January, 1970), the list⁷ of earthquakes in the southern California region (January, 1964 to December, 1971), and the bulletin of the International Seismological Center (January, 1964 to December, 1970). The starting date of January, 1964 was chosen because several of these catalogues were rather incomplete before this date.

No attempt was made to remove aftershocks from these catalogues. We realise that this would be desirable, and attempts to do this are continuing. Some aftershocks are easy to identify, but in many cases it is not at all clear whether an earthquake is an aftershock or not. For the present, we prefer to change the data sets as little as possible.

To make our earthquake time series, we have extracted counts per unit time of earthquakes with magnitudes greater than or equal to a given threshold. The thresholds chosen were high enough to ensure that the catalogues were essentially complete, but low enough that a reasonable number of events were included. For example, in the case of the PDE listing, earthquakes with $m_b < 5.2$ were not used, since there are doubts about the completeness of this catalogue at $m_b \leq 5.0$. Note that the particular definition of magnitude used is immaterial in this representation (for example, the Southern California Catalogue uses local magnitude M_L).

Analysis

Changing this threshold upwards from its minimum value produced only minor changes in the character of the resulting earthquake series. We interpret this as follows: Within a region, the shape of the frequency-magnitude curve seems to be remarkably stable. Its level, however, changes significantly from one time interval to the next. If this relationship is approximated by a straight line

$$\log N = a - bM$$

where N is the number of events with magnitude $\geq M$, and a and b are constants, then b is found to change very little with time. On the other hand, a seems to undergo rapid fluctuations. Clearly, the time series we have constructed give an indication of the variation in the quantity a , which may be called the level of seismic activity.

The choice of the time intervals for the earthquake counts is a little more difficult. Wider intervals contain more events, but also appear to smooth out some high frequency components in the variation of seismic activity. Counts over 40-d intervals were used extensively, and an example is shown in Fig. 1a. In this case shallow (depth < 100 km) earthquakes from subduction zones bordering the Pacific Ocean with $m_b \geq 5.5$ are shown. Some structure is visible in this histogram, particularly in the period 1964–69, but it is still dominated by 'spikes' due to the aftershock sequences of a few large events. We have added more smoothing, by applying a sliding 200-d window to the data, and this is shown in Fig. 1b.

Figure 1b is not very surprising. The years 1964 and 1965 were dominated by the aftershocks of the Alaskan Earthquake (March, 1964) and Rat Island sequence (February, 1965). Activity lows of some significance occurred in mid-1966 and mid-1967, and then the activity returned to a reasonably uniform level for the remainder of the period. The true significance of this figure is not apparent until it is compared with the variations from smaller individual seismic zones.

For a start, let us compare shallow events with deep events. Figure 1c shows the variation for events with depth > 300 km, and $m_b \geq 5.2$, for the whole Pacific area. The depth of 300 km was chosen to ensure a complete separation of deep and shallow events. Remarkably, a high activity in 1964 and 1965 is again shown, falling off to extreme lows in mid-1966 and mid-1967. The remainder of the curve does not correlate in detail with the shallow events. Note that, this time, the 1964–65 activity high can not be attributed to the Alaskan and Rat Island events, since both were shallow. In fact, many of the events in Fig. 1c come from the Fiji-Tonga area.

Figure 2 shows smoothed earthquake time series for six regions bordering the Pacific Ocean. These have been compiled from the PDE catalogue, and contain only shallow events (depth < 100 km). The separation into regions was accomplished using the standard seismic region numbers⁸. If earthquakes really are local phenomena, we would expect essentially no correlation between these time series. Instead, we note that each time series has a high during 1965, though the location of the peak is somewhat variable, and this is followed by distinct activity lows in 1966 and 1967, as found for the Pacific as a whole (Fig. 1). An activity high in 1968 is apparent in most of the time series, and the correlation becomes rather low for the last five years of the record. Clearly, the general features of the first five years of the series shown in Fig. 1b appear in most of these localised regions.

One immediate explanation for these observations might be that they are a characteristic of the PDE catalogue. To check this, Fig. 3 shows the time series for six widely separated areas, using several earthquake catalogues. The areas concerned include subduction zones, mid-ocean ridges and a transform fault (southern California), and the general features of Figs 1 and 2 are again apparent. Notice in particular the remarkable correlation between the top three series of Fig. 3, for the period 1964–68.

Attempts to quantify the correlations between these time series have met with little success. Standard methods of cross correlation tend simply to match the largest peaks, and do not

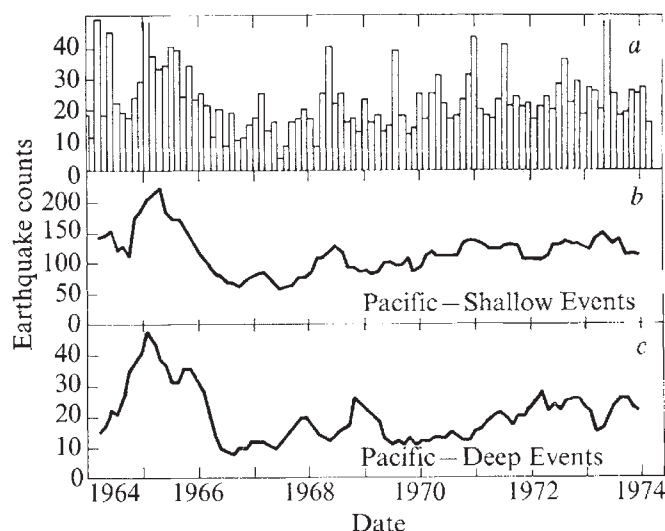


Fig. 1 Earthquake counts for the seismic zones bordering the Pacific Ocean, using the PDE catalogue. *a*, Histogram of shallow (depth < 100 km) earthquakes with $m_b \geq 5.5$ at 40 d intervals. *b*, Smoothed version of the histogram above, using a sliding 200 d window. *c*, Time series of deep (depth > 300 km) earthquakes with $m_b \geq 5.2$, for the same zone, smoothed as in *b*.

emphasise the features apparent to the eye. We are, however, accumulating evidence that the spectra of these time series have some common characteristics, and this will be discussed in a later paper.

Conclusion

The conclusions from the data shown in Figs 2 and 3 are largely self-evident. Seismic activity, even of small earthquakes, contains significant variations in level that are correlatable over large distances, and between different tectonic environments. Viewed as a random process, seismic activity is highly non-stationary. The correlations shown must, however, be interpreted as indicating a strong non-random component.

If very high thresholds for magnitude are used, or if the data are summed to obtain energy release or fault slip, the correlations in Figs 2 and 3 are not apparent. In fact, the most pronounced feature of all, the activity high in 1965 followed by the low in 1966, is not apparent on these curves. We must conclude, therefore, that seismic activity and seismic energy release (or fault slip) are not the same thing.

We can account for these observations by invoking the concept of a triggering stress. We postulate that tectonic plates are subject to stresses of several different origins, with different amplitudes and spectral characteristics. There must be a long term stress accumulation due to the basic mechanism of plate motion, presumably as a result of processes in the Earth's interior. Superimposed on this, however, will be a variety of shorter term, often periodic, stresses. External effects such as changes in the rate of rotation of the Earth⁹ and tidal stresses must be important here. The net sum of all these is a fluctuating stress that is generally of rather low amplitude (almost certainly

Fig. 2 Earthquake time series for six regions on the margins of the Pacific Plate. Magnitude thresholds vary from $m_b = 5.2$ to $m_b = 5.6$. Source: PDE earthquake catalogue.

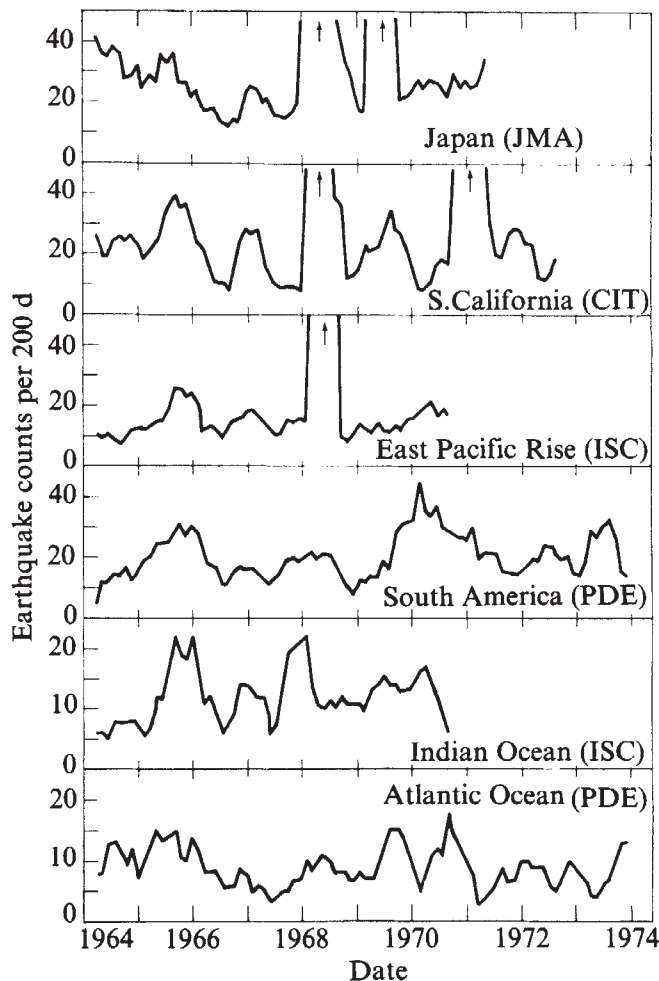
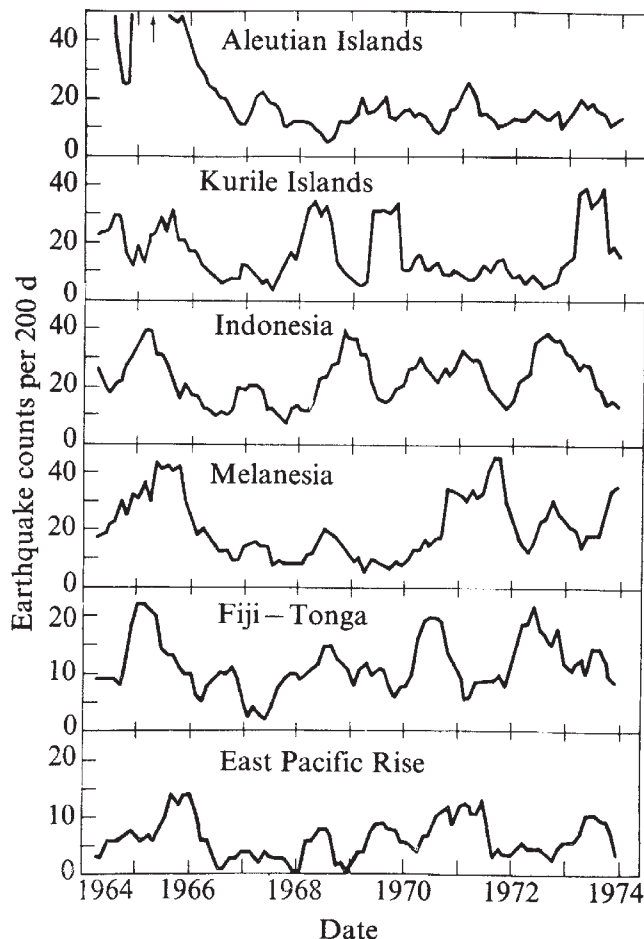


Fig. 3 Earthquake time series for six widely separated areas. Sources and magnitude thresholds are as follows: JMA, Japan Meteorological Agency Catalogue ($m_b \geq 5.2$); CIT, Southern California Network Catalogue ($M \geq 3.5$); ISC, International Seismological Center Bulletin ($m_b \geq 4.8$); PDE, PDE earthquake listing ($m_b \geq 5.2$).

$< 10^5$ Pa (1 bar)) but which will change in a complicated way.

Such a fluctuating stress may be expected to trigger an earthquake in any area where the ambient stress is very close to failure. In most cases, only small earthquakes will be triggered. Occasionally, however, the small increment in stress will trigger a large earthquake, though there may be little relation between the size of the stress change and the size of the triggered event. This is presumably the reason why seismic energy release seems to be a quasi-random process.

It is also conceivable that this triggering stress may very occasionally become quite large, when the various contributions to this stress happen to reinforce one another. At such a time one might expect a widespread triggering of seismic activity, including a number of large events. This is essentially the hypothesis that Anderson⁹ has proposed to account for the strong increase in seismic activity that occurred about the year 1900. Perhaps a similar explanation may apply to other periods of unusual activity, such as the year 1755, when large earthquakes occurred in Central America, Persia, Iceland, Lisbon, Morocco and Switzerland, and New England experienced the largest earthquake in its historical record.

The implications of this hypothesis are important. If it is true, then to investigate the nature of the triggering stresses we should be studying the occurrence of small earthquakes, not large ones. The level of seismic activity, defined by the level of the frequency-magnitude curve, may be giving us a direct measure of this fluctuating applied stress. This may lead to a

whole new approach to the problem of earthquake prediction.

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Evolutionary size increase and longevity in Jurassic bivalves and ammonites

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A study of the relationship between the rate of increase of size and generic or specific longevity in Jurassic ammonites and bivalves shows that taxa which increase more rapidly become extinct more quickly. The results, which can be interpreted stochastically, make it possible to distinguish two modes of evolution respectively involving an increase and decrease in size.

THERE is a common tendency recognisable in the fossil record for animal groups to have evolved towards larger size; it is generally known as Cope's Rule¹⁻³. No systematic attempt has, however, been made to investigate any possible relationship between the rate of size increase for particular taxa and their duration in time, or longevity. I report here on a study of the relationship between size increase and longevity for two of the most abundant macroinvertebrate groups in the classic marine Jurassic deposits of western Europe, the bivalves and ammonites (see refs 4-10). Cope's Rule is well exemplified by both fossil groups. Full allowance has been made for ecological control of size in different facies, and there is no evidence of a secular environmental trend through the Jurassic which could have affected the size attained by particular taxa at successive horizons¹¹. Consequently, there can be little doubt that the size changes represent a genuine evolutionary phenomenon.

A good unit to use in measuring the rate of size increase is the darwin, proposed by Haldane¹² as a unit of evolutionary rate. One darwin is strictly defined as a change of e in a million years, but Haldane treated this as practically equivalent to an increase or decrease of $10^{-3}/10^3$ yr, that is, a doubling or halving of size in 1×10^6 yr. The latter definition is adopted here.

Results

Data for the best documented bivalve species and genera (more strictly, species groups within genera) are presented in Table 1, which records the maximum dimension attained by each taxon at the earliest and latest stratigraphic occurrence, to the nearest zone, together with the longevity in zones and a measure of the rate of size increase. (The average duration of a Jurassic zone is approximately 1×10^6 yr (ref. 11).) The mean rate of size increase is 109 mdarwin (range 6-546), which accords well with the values established by Van Valen¹³ in a general survey of invertebrates and protists. He found a range of 3-300, with most values clustering between 30 and 150. The longevity ranges from 3 to 60 zones, the latter range representing almost the whole duration of the Jurassic Period.

For the ammonites, which have been excessively split taxonomically, the genus is generally the lowest reliable

taxonomic unit, but Table 2 includes data for a few well defined species. The excessive splitting means that certain closely-related forms, which in other groups would almost certainly be put in the same genus, are given different generic names. For that reason several examples, for instance, *Grammoceras-Phlyseogrammoceras*, are included in Table 2. Because the stratigraphic range of most genera is so short adequate data are more difficult to obtain than for bivalves, so the number of examples is less.

There is little doubt that evolutionary size increase is more common than indicated by Table 2. For instance, Zeiss¹⁴ records it as a common trend in Tithonian ammonities, though he fails to provide usable data. Because of the short time range size data have been determined where possible down to subzonal level. The mean value of 584 mdarwin, though significantly higher than that for the bivalves, is still probably too low, because the data are somewhat biased in favour of long-ranging taxa such as the oppeliid genera *Glochiceras*, *Ochetoceras* and *Taramelliceras*, which have relatively low rates of size increase. Whereas the zonal range is 1/3-15, the great majority of Jurassic ammonite genera have a stratigraphic range of about three zones or less.

When S , the rate of size increase, is plotted against L , the longevity, a strongly curvilinear relationship is exhibited for the two groups. Where the results are replotted logarithmically, the points cluster closely about a straight line (Fig. 1). Ammonites and bivalves, genera and species, large and small fossils do not form discrete groups but overlap in their distribution. It would give a spurious air of accuracy to calculate a regression and correlation coefficient, because the time scale is, of necessity, only approximate. It is, however, evident that the correlation is good and a regression line can easily be fitted by inspection. This indicates a relationship expressed by the equation

$$S = bL^{-a}$$

where a is the slope and b the intercept. The slope, a , is almost unity, and, if S is expressed in darwins, b is exactly unity. Therefore, the relationship between the ratio of size increase and longevity closely approximates to a hyperbolic function, $S = 1/L$.

This equation can be applied to the one result which cannot be plotted on the logarithmic scale, the ammonite *Metophioceras conybeari*, which has the shortest range and highest millidarwin value of all. The data for this species are based on examination in the field and can be considered relatively reliable. The rate of size increase, S , = 3.69 and $1/L$ = 3.33, which are satisfyingly close. The ammonite genus *Pleuroceras* has an abnormally high millidarwin value for its longevity of one zone. This could be attributable to several factors. The zone in which it occurs could have been of shorter than average duration, the minimum and maximum sizes may not have been separated in time by the whole duration of the zone, or the minimum

Table 1 Bivalve data

Taxon	Age range	Number of zones	Increase in maximum size (mm)	mdarwin
<i>Astarte elegans</i> (1)*	M.Toar.-L.Baj.	13	26-62	92
<i>A. gueuxi</i> (1)	U.Het.-L.Plien.	8	20-38	119
<i>Barbatia pectinata</i> (1)	L.Baj.-U.Oxf.	21	42-60	34
<i>Camptonectes lohbergensis-lamellosa</i> (1, 3)	L.Plien.-Tith.	55	23-109	43
<i>Cardinia concinna</i> (1, 2)	U.Het.-U.Plien.	11	45-157	159
<i>Ceratomyopsis striata</i> (1, 2, 4)	U.Bath.-L.Kim.	18	58-90	43
<i>Chlamys textoria</i> (1, 2)	L.Sin.-U.Plien.	10	28-88	157
<i>C. (Radulopecten) fibrosa-inaequicostata</i> (1, 2)	L.Bath.-U.Oxf.	14	37-60	58
<i>Coelopsis lunulatus</i> (1)	U.Toar.-L.Baj.	6	32-79	206
<i>Ctenostreon tuberculatum-pectiniforme</i> (1, 2, 3)	L.Sin.-U.Tith.	60	58-212	30
<i>Entolium lunare</i> (1)	U.Sin.-U.Plien.	8	34-110	202
<i>Eocallista antiopa-brongniarti</i> (1, 3, 4)	U.Bath.-L.Kim.	18	28-90	89
<i>Eonavicula minuta-quadrisculata</i> (1, 3)	U.Bath.-L.Tith.	23	21-69	71
<i>Eopecten velata</i> (1)	U.Het.-U.Plien.	11	102-153	68
<i>Eopecten abjecta</i> (1, 2)	U.Aal.-U.Oxf.	24	75-112	31
<i>Gervillella lanceolata-aviculoides</i> (1, 2)	L.Sin.-U.Oxf.	40	60-262	55
<i>Goniomya hybrida-literata</i> (1)	U.Plien.-U.Oxf.	33	48-90	28
<i>Grammatodon insons</i> (1)	L.Sin.-L.Plien.	8	19-56	187
<i>G. insons-oblonga</i> (1, 3)	L.Sin.-L.Baj.	20	19-106	83
<i>Gryphaea arcuata-gigantea</i> (1, 3)	U.Het.-U.Plien.	11	60-125	90
<i>G. bilobata-dilatata</i> (1, 2)	U.Toar.-M.Oxf.	26	70-137	40
<i>Isocyprina menkei-dolabra</i> (1)	U.Sin.-L.Baj.	19	21-56	70
<i>Isognomon lugdunensis-myltiloides</i> (1, 2)	U.Plien.-L.Kim.	40	67-180	34
<i>Liostraea irregularis</i> (1)	L.Het.-U.Plien.	13	30-133	116
<i>Modiolus hillanus-imbricatus</i> (1)	L.Het.-L.Baj.	25	45-150	67
<i>Myoconcha decorata</i> (1)	L.Sin.-U.Plien.	10	51-108	106
<i>M. decorata-crassa</i> (1, 3)	L.Sin.-L.Baj.	21	51-145	68
<i>Myophorella signata-clavellata</i> (1, 2)	L.Baj.-L.Kim.	28	91-145	28
<i>Palaeonucula navis-hammeri</i> (1, 3)	L.Plien.-Aal.	11	8-29	165
<i>Paralleodon buckmani</i> (1)	L.Het.-L.Plien.	10	24-41	85
<i>P. hirsoneensis</i> (1)	U.Toar.-L.Baj.	6	45-100	180
<i>Pholadomya ambigua</i> (1, 2)	L.Sin.-U.Plien.	10	68-150	110
<i>P. lirata-protei</i> (1, 3)	L.Baj.-U.Oxf.	22	78-108	31
<i>Plagiostoma gigantea-hersilia</i> (1, 2)	L.Het.-L.Baj.	21	100-207	39
<i>Plicatula laevigata</i> (1)	L.Plien.-U.Plien.	3	63-85	225
<i>Propeamussium pumilum-laeviradiatum</i> (1)	L.Toar.-L.Aal.	6	8-30	313
<i>Protocardia phillipiana-dissimilis</i> (1)	U.Sin.-L.Tith.	56	16-90	16
<i>Pseudomytiloides dubius</i> (1, 3)	L.Toar.-U.Toar.	5	30-45	150
<i>Pseudopecten aequivalvis</i> (1, 3)	L.Plien.-U.Plien.	3	61-200	546
<i>Ryderia graphica</i> (1)	L.Sin.-L.Plien.	8	14-38	170
<i>Thracia depressa</i> (1, 2, 4)	U.Toar.-U.Kim.	40	40-94	29

* Numbers in parentheses indicate location of specimens measured: (1), British Museum (Natural History); (2), Museum National d'Histoire Naturelle, Paris; (3), Palaeontological Institute of Tübingen University; (4), Palaeontological Institute of Göttingen University.

Jurassic stages: Het., Hettangian; Sin., Sinemurian; Plien., Pliensbachian; Toar., Toarcian; Aal., Aalenian; Baj., Bajocian; Cal., Callovian; Oxf., Oxfordian; Kim., Kimmeridgian; Tith., Tithonian. L., Lower; M., Middle; U., Upper.

Table 2 Ammonite data

Taxon	Age range	Number of zones	Increase in maximum size (mm)	mdarwin
<i>Arnioceras semicostatum</i> (1)*	L.Sin.	4	55-91	207
<i>Aulacostephanus</i> ¹⁰	L.Kim.	3	175-345	328
<i>Caenisites-Asteroceras</i> (1)	Sin.	2	147-270	459
<i>Cardioceras</i> (1)	L.Oxf.	2	137-210	383
<i>Coroniceras-Primarietites</i> (1)	L.Sin.	1	350-650	928
<i>Creniceras dentatum</i>	L.Kim.	3	20-37	308
<i>Garantiana-Parkinsonia</i> (1)	U.Baj.	2	110-350	785
<i>Glochiceras</i> (3)	U.Oxf.-U.Kim.	10	39-56	64
<i>Grammoceras-Phylseogrammoceras</i> (1)	U.Toar.	2	114-194	425
<i>Idoceras</i> (3)	U.Oxf.-L.Kim.	5	154-221	143
<i>Kosmoceras (Gulielmites)</i> ⁴	M.Cal.	1	70-150	1,071
<i>Leioceras</i> ⁷	Aal.	1	60-100	833
<i>Liparoceras</i> ⁸	L.Plien.	3	88-285	540
<i>Metophioceras conybeari</i> (1)	L.Sin.	1/3	130-320	3,690
<i>Ochetoceras</i> (3)	U.Cal.-U.Kim.	15	79-134	57
<i>Pectinatites</i> ⁵	L.Tith.	3	184-380	206
<i>Pleuroceras</i> ⁶	U.Plien.	1	66-335	2,538
<i>Psiloceras</i> (1)	L.Het.	1	130-253	973
<i>Taramelliceras</i> (1, 3)	L.Oxf.-L.Kim.	12	50-196	165

* Numbers in parentheses as for Table 1.

value, may be an underestimate, either because of inadequate sampling or because of a failure of preservation of the outer body chamber, which is not unusual among ammonites. These points only serve to emphasise the importance of very careful assessment of the evidence by specialists, and do not detract from the overall close correlation exhibited by Fig. 1.

That one parameter is so near to the reciprocal of the other is a remarkably simple result, especially bearing in mind the approximation of the time scale. The result is only in part an artefact of the method of plotting. By necessity the estimation of S involves using the reciprocal of L , but the amount of size change in time is an independent variable (in other words, the plot is not simply of one parameter against its reciprocal). If there were no such simple relationship as claimed between S and L there would be a much wider scatter of points in the graphical plot. The size increase that has taken place within individual taxa can be considerable. Thus a majority, almost the same in both groups (69% of bivalves, 68% of ammonites) have increased in size by a factor of approximately two or more, and a significant minority have trebled, quadrupled or even quintupled in size. On the other hand, a size increase of appreciably less than twofold is readily recognisable in the stratigraphic record.

Another possible criticism is that the results portrayed are selective, because they relate only to a minority of taxa in the European Jurassic. Though they are admittedly restricted to those taxa which seem to exemplify Cope's Rule, further work on abundant and precisely dated material may well show that the rule applies more widely. This is especially true for the ammonites, where a neglect of the significance of size differences has in the past influenced the classification of taxa, as is evident from the widespread failure until recently to recognise sexual dimorphism, with different names being given to the macroconchs and microconchs.

Before an interpretation is attempted some more facts should be considered. Figure 2 shows the nature of the size increase

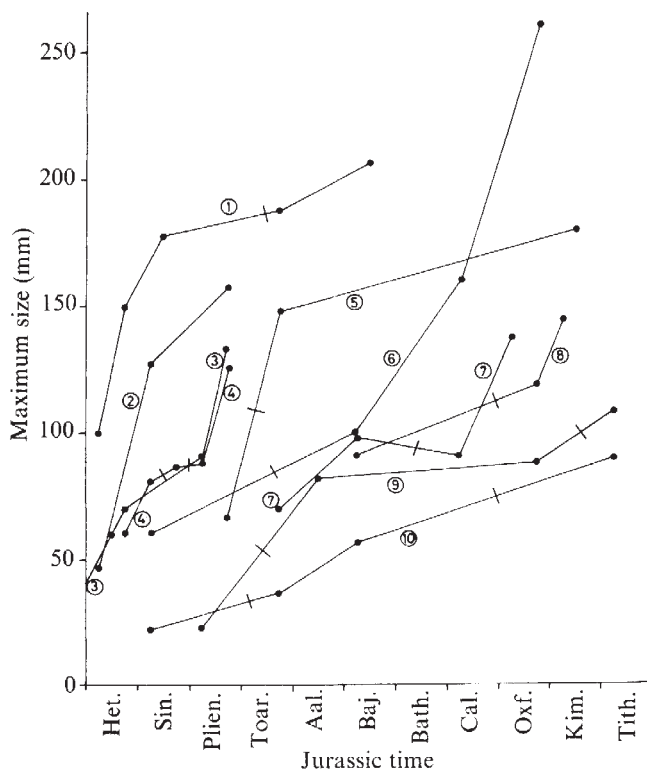


Fig. 2 Pattern of size increase during the Jurassic period for selected bivalves (stage abbreviations as in Tables 1 and 2). 1, *Plagiostoma*; 2, *Cardinia concinna*; 3, *Liostrea irregularis*; 4, *Liassic Gryphaea*; 5, *Isognomon*; 6, *Gervillella*; 7, Middle-Jurassic *Gryphaea*; 8, *Myophorella*; 9, *Camptonectes*; 10, *Protocardia*. Cross bars indicate change of species or subspecies.

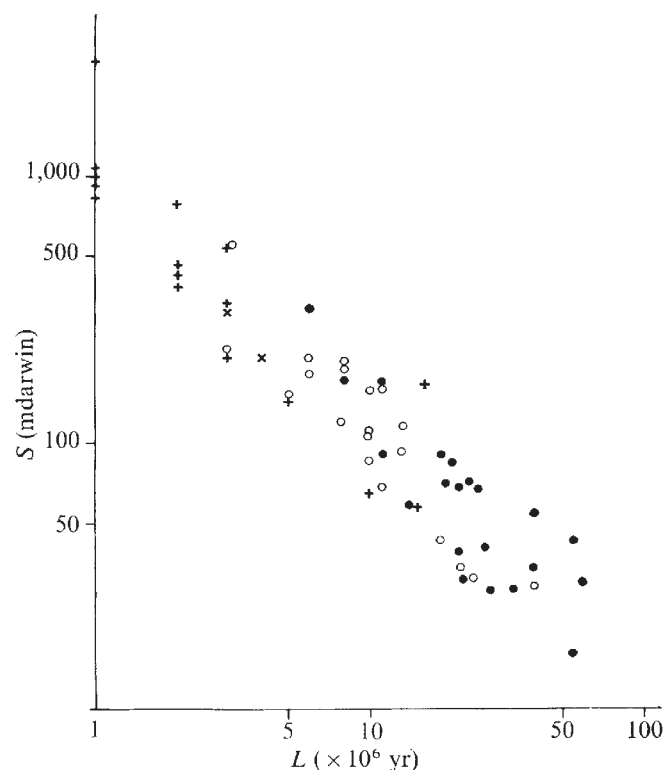


Fig. 1 Logarithmic plot of rate of size increase, S , against longevity, L , for Jurassic bivalves and ammonites. +, Ammonite genera; x, ammonite species; ●, bivalve genera; ○, bivalve species.

for a sample of longer-ranging bivalves where several data points are available, to illustrate the range of variation in pattern. Some forms (*Plagiostoma*, *Isognomon*, *Cardinia*) show a decrease in rate of increase with time, others an increase (*Gervillella*, *Myophorella*), or something close to a linear relationship (*Protocardia*). Others are more complicated, as, for instance, *Gryphaea*, for which there are more data available^{15,16}. The early Jurassic evolutionary lineage *G. arcuata-mccullochii-gigantea* shows successively a decrease in rate followed by an increase, whereas the Middle to Upper Jurassic lineage *G. bilobata-dilatata*, starts at a much smaller size than the end member of the older lineage, then increases, then decreases slightly, and finally increases again. These changes in rate of size change and absolute size correlate with taxonomic change, the new taxa, species or subspecies, sometimes being smaller than the taxa from which they evolved.

This illustrates an important principle; in both bivalves and ammonites younger taxa are often appreciably smaller than their evolutionary precursors, but with no discernible size transitions from one to the other. Good examples are, among the bivalves, the genera *Entolium*, *Gryphaea*, *Liostrea*, *Plagiostoma*, *Pholadomya* and *Eoplectena*; among the ammonites the lineages *Psiloceras-Waehneroceras-Schlotheimia*, *Amaltheus-Pleuroceras*, and *Quenstedtoceras-Cardioceras-Amoeboceras*. A further point to note is that, because of adaptive radiation, both small and large species of the same genus may coexist in the younger part of the stratigraphic range. Close analysis is required, by ammonite specialists in particular, of whole phyletic lineages in uncondensed stratigraphic successions, in terms of times of morphological divergence and progressive size change. Although generally the ammonite genera may exhibit Cope's Rule, in some cases only the species may show it, assuming they are sufficiently well defined (compare with *Gryphaea*).

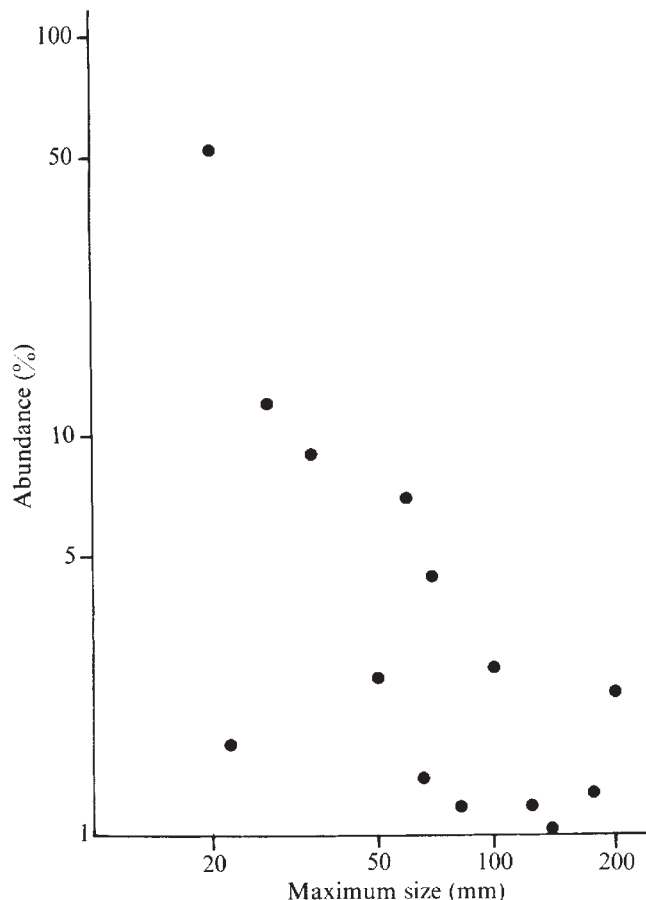


Fig. 3 Logarithmic plot of maximum size against abundance for the 14 most abundant species in the English Corallian Group.

Interpretation

Though ammonites and bivalves have evolved at markedly different rates, probably because of varying intensities of inter-specific competition¹⁷, they seem to obey the same rule relating rate of size increase to longevity. Since phyletic size increase is such a widespread trend in the animal kingdom there must manifestly be one or more selective advantages of larger size. Among those proposed¹⁻³ are: an improved ability to capture prey or ward off predators; a greater reproductive success; increased intelligence (with greater brain size); increased regulation of the internal environment; decreased annual mortality; and increased heat regulation per unit volume. Odum¹⁸ considers that what he calls antithermal maintenance is the main priority in any complex system such as an organism, and this is decreased per unit of biomass structure if the individual's size is large. Stanley³ considers that Cope's Rule should be viewed in terms of the evolution of small forms towards a larger optimum size for the animal group in question. It is not counterbalanced by an 'anti-Cope's Rule' because large animals of a given group are almost invariably too highly specialised to give rise to new forms, whereas many small animals are relatively unspecialised.

The acceptance of Stanley's interpretation of Cope's Rule implies that organisms that increase in size more rapidly should become extinct more quickly because extreme specialisation would occur earlier, thus rendering them more vulnerable to extinction. This may be termed the deterministic hypothesis which, though it may well be true in particular cases, suffers from the drawback that it assumes what requires to be proved. Too little is known of the mode of life of the extinct ammonites, or even the bivalves for that matter, for such an hypothesis to be accepted without considering an alternative.

Another, probabilistic, hypothesis can, however, be proposed. We may accept that selection pressures will tend to promote size increase, at least up to an optimum for a particular group, because of the manifest advantages already outlined. A 'price', as it were, has to be paid, however. Because food resources are likely to remain approximately constant, population sizes must decrease, thereby increasing the probability of extinction. Thus organisms that increase in size more rapidly should become extinct sooner. (The assumption that rarer animals are more likely to become extinct has received full theoretical treatment by McArthur and Wilson^{19,20}.)

The proposition that larger animals are rarer than smaller is abundantly supported by common observation. It is just as true within a particular group as between for instance, elephants and flies. Fürsich (personal communication) has made a systematic collection from all facies types of specimens from the English Corallian Group (Oxfordian Stage). Figure 3 shows a double logarithmic plot of abundance against maximum size for the 14 most abundant species (a total of 13,355 specimens). The logarithmic plot shows a closer resemblance to a linear relationship than a direct plot, which is suggestive of a power function. The scatter is inevitably considerable, however, which is not at all surprising. It would only be negligible if the relative abundance for a given size were constant, and there is no reason why that should be so. A best fit curve would have the advantage, in fact, of enabling one readily to distinguish forms which were more or less abundant than average for their size. The important point to be stressed is that these results give quantitative expression to the marked decline in numbers with increasing size. The smallest species, *Nanagyra nana*, accounts for 52% of the total number of individuals whereas the five largest species account together for a mere 8%.

The extraordinary, and unexpected, simplicity of the relationship between the rate of size increase and longevity, bringing under one umbrella large and small species and genera of two biologically very different molluscan groups, provides support for the stochastic interpretation.

In conclusion, two distinct modes of evolution can be proposed. One is more or less gradualistic and involves increase in size independent of changes in environment, which is generally speaking an evolutionary dead end, since it leads to eventual extinction, commonly without further phenotypic modification. It is a continuous unilinear trend involving orthoselection. The other involves the abrupt appearance of descendant species smaller than their ancestors: I know of no examples of gradual size decrease recognisable in the stratigraphic record, either in Jurassic molluscs or any other fossil group. Yet, undoubtedly, small organisms have evolved from larger, but the process must have been relatively sudden and probably involved small populations, so that the chances of finding intermediates in the fossil record is minimal. The process probably often involves pedomorphosis, and may be the principal means by which new morphotypes arise.

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Molecular structures of cytidine-5'-diphosphate and cytidine-5'-diphospho-choline, and their role in intermediary metabolism

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The nucleotide coenzyme cytidine-5'-diphospho-choline is highly folded. The CMP-5' parts of the molecules in the crystal structure are strongly linked by metal ligation and hydrogen bonds leaving the phosphoryl-choline residues relatively free. Cytidine-5'-diphosphoric acid exists as a zwitterion with N31 protonated. The P-O bond lengths from the anhydride bridging oxygen in the pyrophosphate are significantly different.

NUCLEOSIDE-5'-DIPHOSPHATES have an essential role in many metabolic processes and are the starting substances for the biosynthesis of a variety of activated compounds of the type base-ribose-P-O-P-base (or sugar) involved in the synthesis of many key biological molecules. The presence of the pyrophosphate linkage, important for the activity of many 'high energy' molecules adds further significance to the structures of these molecules. No nucleoside diphosphate structure has, however, been reported so far, probably because of difficulties in growing crystals suitable for single crystal X-ray analysis. (A short report on the rubidium salt of ADP has appeared; F. A. Muller and A. B. Deluke, ACA Meeting, 1971; no structural details are, however, available.) To date the only nucleotide with pyrophosphate linkages to have been solved is the sodium salt of ATP¹. We have now crystallised several of these compounds and have begun a systematic study of their conformations by X-ray analysis. Conformational differences among these are of interest as they behave differently in similar biochemical reactions. The group transfer reactions involving UDP sugars, for example, occur with the cleavage of the glucosyl-phosphate bond, whereas those involving CDP occur with the cleavage of the pyrophosphate linkage. X-ray studies of these molecules can be expected to provide information on those structural features of the molecules which relate to their biological function just as the structural studies of nucleoside-5'-monophosphates have now given a wealth of information relevant to the conformation of nucleic acids. Here we present the results of our study on the structures of cytidine-5'-diphosphoric acid (CDP) and cytidine-5'-diphospho-choline. CDP derivatives are key intermediates in the metabolism of phospholipids. CDP-choline, in particular, is involved in the biosynthesis of lecithin and sphingomyelin and is also important in the formation of plasmalogen in liver and brain².

The two structures show several interesting features. The molecular parameters which contribute to the structural integrity of nucleoside-5'-monophosphates, namely the *gauche-gauche* conformation about the C4'-C5' bond and the *anti* conformation about the glycosidic linkage are preserved in the nucleoside-5'-diphosphates. The pyro-

phosphate orientations with respect to the furanose ring are, however, strikingly different and are related as right and left rotations about the terminal ester bonds of the pyrophosphate diester bridge. The CDP-choline molecules are highly folded and bind to each other so that the CMP-5' parts are rigidly linked by metal ligation and hydrogen bonds. In contrast, the phosphoryl choline groups are loosely held in the crystal structure by water molecules. Such association of coenzyme molecules may influence enzymatic reactions where the phosphoryl choline moiety is transferred leaving behind CMP-5'. The present structures provide for the first time the detailed geometry of the diester pyrophosphate linkage essential for the activity of many other important nucleotide coenzymes like UDP-glucose, nicotinamide adenine dinucleotide (NAD), flavine adenine dinucleotide (FAD) and coenzyme A, the X-ray structures of which have not yet been determined. On the basis of this geometry it now seems possible³ to put forward rational molecular models for some of these molecules.

Experimental details

The CDP-choline used in this investigation was obtained as a monosodium salt from Sigma and the CDP free acid from Boehringer Mannheim. The crystals were grown by slow diffusion of acetone-alcohol mixtures into aqueous solutions of the samples, a technique found successful earlier in growing crystals of nucleoside-5'-monophosphates⁴. Crystallisation was carried out at about 5 °C to avoid possible breakdown of samples by hydrolysis. The CDP-choline crystals were predominantly platy and cleaved easily when allowed to dry fast. They were also strongly hygroscopic. All X-ray measurements were carried out on crystals sealed inside Lindemann capillary tubes along with mother liquor. In contrast CDP free acid grew as long needles and was quite stable in air. Cell constants were initially measured from Weissenberg photographs and subsequently on a Picker 4-circle diffractometer (Table 1).

The X-ray intensities were measured on a diffractometer, using copper K α radiation, up to a 2θ maximum of 127° giving a resolution of 0.86 Å. There was no significant

Table 1 Crystal data

	CDP-choline	CDP free acid
Molecular formula	C ₁₄ H ₂₆ O ₁₁ N ₄ P ₂ Na ₅ H ₂ O	C ₈ H ₁₃ N ₃ O ₁₁ P ₂ H ₂ O
Molecular weight	601.4	421.2
Space group	P2 ₁ 2 ₁ 2 ₁	C2
Z	4	4
a	29.434 Å	27.166 Å
b	12.436 Å	5.566 Å
c	7.016 Å	10.742 Å
β	—	99.99°
Volume	2568.15 Å ³	1599.63 Å ³
d_{measured}	1.57 g ml ⁻¹	1.745 g ml ⁻¹
$d_{\text{calculated}}$	1.56 g ml ⁻¹	1.749 g ml ⁻¹
$\mu(\text{CuK}\alpha)$	43.6 cm ⁻¹	30.1 cm ⁻¹

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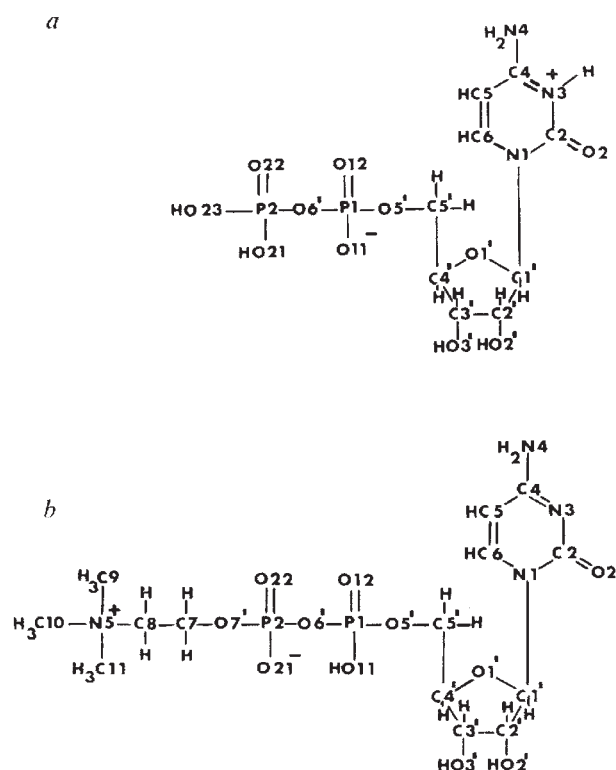


Fig. 1 Atom numbering scheme for: *a*, CDP; *b*, CDP-choline.

decrease in the intensities of monitor reflections over the course of the data collection (about 2 weeks). The total number of significant independent reflections was 1,398 for CDP and 1,439 for CDP-choline, to which the usual corrections other than absorption were applied. Full experimental details will be published elsewhere.

Solution of structure

The positions of phosphorus atoms in both structures were located from sharpened Patterson maps and Harker sections. The structure of CDP was solved by the application of vector superposition techniques and difference Fourier syntheses as direct methods were not successful. Application of magic integers³ and tangent refinement (locally programmed by Dr G. M. Sheldrick) aided the complete solution of CDP-choline. The structures were refined by full-matrix least squares calculations with anisotropic temperature factors to current *R* factors of 9.1% (CDP-choline) and 6.3% (CDP). The contributions from hydrogen atoms associated with the nucleotide molecules were included but water hydrogens could not be located due to high temperature factors of the water oxygen atoms.

Description of structures

The chemical structures and the numbering schemes adopted for the two compounds are shown in Fig. 1.

Nucleoside geometry. The orientation of the cytosine base with respect to the sugar is *anti* in both molecules, the glycosidic torsional angle χ_{CN} about the C1'–N1 bond being

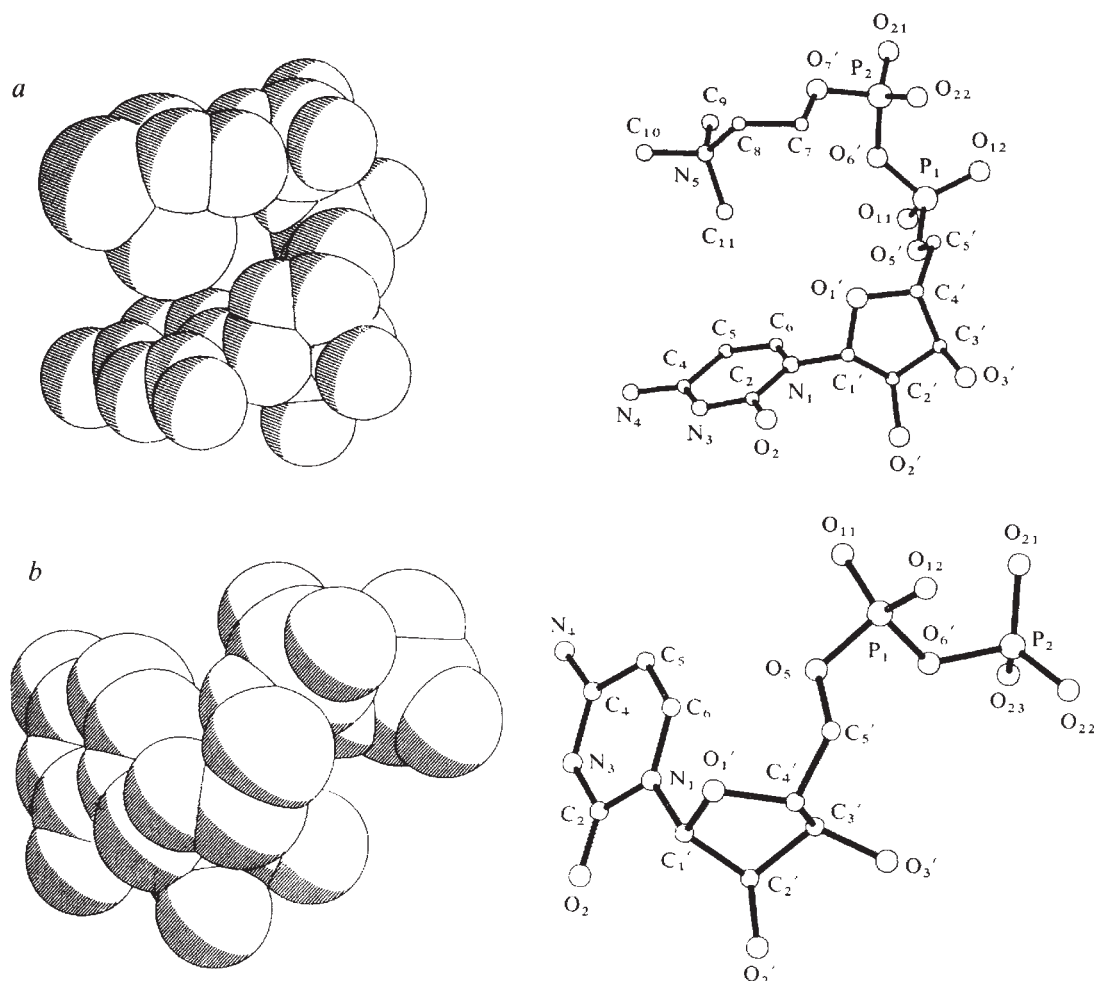
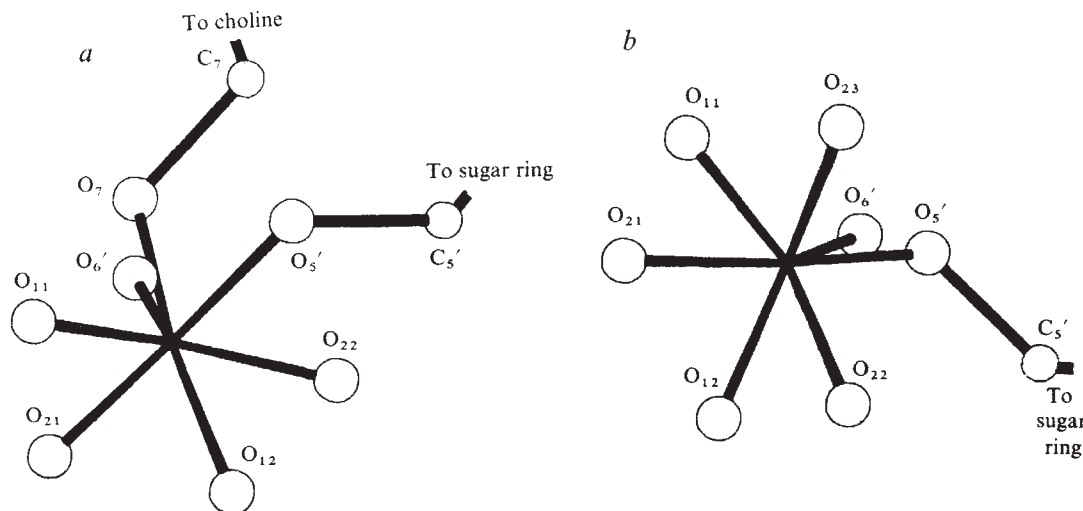


Fig. 2 Space filling and perspective drawings for: *a*, CDP-choline; *b*, CDP.

Fig. 3 View down the P-P vector: *a*, CDP-choline; *b*, CDP.



22.9° (CDP) and 60.35° (CDP-choline), (for notations see Sundaralingam⁶). The ring nitrogen N3 in the free acid is protonated forming a cytosyl cation, due to single proton migration from the phosphate group. Earlier structure determinations of cytidine-3'-phosphate^{7,8} and deoxycytidine-5'-phosphate⁹ have established that cytosine nucleotides prefer the zwitterion structure. The bond lengths and angles of the base in CDP and CDP-choline correspond to those one expects for N3 protonated and neutral cytosine bases, respectively⁹.

In CDP, the best four atom plane through the furanose ring passes through C4'-O1'-C1'-C2'. The C3' atom is displaced from this plane by 0.63 Å on the same side as C5'. The sugar conformation is therefore C3'-*endo*. In CDP-choline, however, the sugar shows a typical C2'-*endo* puckering with C2' displaced from the four-atom best plane by 0.57 Å. The distribution of glycosyl torsional angles χ_{CN} and their relationship to the nature of ribose puckering in the known β -pyrimidine glycosides shows^{9,10} that C2'-*endo* puckering favours $36^\circ \leq \chi_{CN} \leq 65^\circ$ whereas C3'-*endo* puckering favours $0^\circ \leq \chi_{CN} \leq 42^\circ$. Note that the steric relationship observed in the nucleosides and their monophosphates is also the one found in the present diphosphate structures. In CDP-choline the cytosine base is almost perpendicular to the sugar ring, the dihedral angle between the normals to their mean planes being 92.81° . This angle in CDP is 107.02° . For each compound the conformation about the exocyclic C4'-C5' bond is *gauche* with respect to both C4'-O1' and C4'-C3' bonds with the torsional angles: O1'-C4'-C5'-O5' = -60.18° (CDP) and -63.55° (CDP-choline) and C3'-C4'-C5'-O5' = 55.9° (CDP) and 57.44° (CDP-choline). An earlier survey of X-ray crystallographic results has led Sundaralingam¹¹ to propose that 5'-nucleotides are far more rigid than nucleosides, the conformation about the C4'-C5' bond being *gg* whereas nucleosides exhibit all the three possible staggered conformations—*gg*, *gauche trans* (*gt*) and *trans-gauche* (*tg*). The only exceptions so far are 6-azauridine-5'-phosphate¹² and deoxyguanosine-5'-phosphate^{13,14} which have *gt* conformation. The present studies show the general validity of Sundaralingam's proposal even in case of nucleoside (pyrimidine)-5'-diphosphates.

Pyrophosphate conformation

The pyrophosphate group both in CDP and CDP-choline has the characteristic staggered conformation. The bond lengths of the bridging O6' to the two phosphorus atoms show significant differences: CDP: P1-O6' = 1.58, P2'-O6' = 1.62 Å, P1-O6'-P2 = 127.8° ; CDP-choline: P1-O6' = 1.60, P2-O6' = 1.64 Å, P1-O6'-P2 = 131.7° . This suggests that the two halves of the diphosphate have different resonance

hybrid states. The differences in the bond lengths are more pronounced than those found in the only other organic pyrophosphates so far solved¹⁵⁻¹⁷, namely di- β -naphthyl pyrophosphate¹⁵ (P1-O6' = 1.59, P2-O6' = 1.61 Å) and thiamine pyrophosphate¹⁶ (P1-O6' = 1.58, P2-O6' = 1.58 Å). An interesting feature of the CDP and CDP-choline molecules is that while the pyrophosphates in both have the staggered geometry, their orientations in the molecules are strikingly different. The P-P vectors correspond to left and right rotations about the C5'-C4' bond, the diphosphate group oriented towards the ribose hydroxyl oxygens in CDP and away from them in CDP-choline. It is as if the second phosphate in CDP-choline is linked with O11 as the bridging oxygen instead of O6'. These features are shown in Fig. 2.

CDP-choline molecule has a folded shape

The present analysis is the first one to give information about the conformation in the solid state of a nucleotide containing the pyrophosphate diester linkage. The geometry of this group and the choline moiety have features which lead to a highly folded shape. Figure 3 shows the molecules viewed down the P-P vector. The three fundamental angles O6'-P2-O7'-C7, P2-O7'-C7-C8 and O7'-C7-C8-N5 are 72.7, -166.7° , and 68.1° and the choline moiety has thus the *trans near-gauche* conformation. This preferred energy conformer was found both in the crystal structures of choline derivatives^{18,19} and in minimum potential energy computations²⁰ of the O-C-C-N⁺ system. The only other crystal structures containing the phosphoryl-choline moiety which have been analysed are those of glyceryl phosphoryl-choline²¹ and its cadmium chloride complex²², where similar conformations were found.

Interactions between molecules

Hydrogen bonding and other interactions between nucleotide bases, like and unlike, are fundamental to their recognition and function in biological systems. In the two structures reported here the cytosine base exists in two different tautomeric forms; protonated (CDP) and neutral (CDP-choline). Their interactions in the crystal structures are shown in Fig. 4.

As can be seen in CDP-choline, the imidine nitrogen N3 prefers, in the presence of the metal ion, to form an ionic bond rather than a hydrogen one. The ribose hydroxyl atoms also show a similar pattern. While they are involved in metal ligation in CDP-choline, in CDP they form strong hydrogen bonds with 2₁ screw hydroxyl oxygens. This hydrogen bonding network has a prominent role in the

of the reaction. On the assumption that enzyme recognition is influenced both by specific shape and substrate-substrate bonding, our experimental observations on the crystal structure of CDP-choline may well have relevance to the *in vivo* action of this coenzyme. The folded shape of the molecules with CMP-5' parts strongly bound and the phosphoryl-choline groups only loosely held permits easy cleavage of the pyrophosphate bond facilitating the direction of such a reaction.

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letters to nature

Nova in Cygnus of record brightness

THE recent bright nova in Cygnus is probably one of the most remarkable galactic novae observed. Invisible on the Palomar Sky Survey it had climbed through at least 18 mag to its maximum of $V = 1.8$ on August 31.0 (UT). This led to some early speculations whether this might be the first galactic supernova observed since Kepler's days. This suggestion is not, however, supported by the spectrum of the object and is also inconsistent with the extremely rapid decline in brightness after maximum. On the other hand, assuming it to be a classical nova, there are indications that it is a very luminous one.

Using the 61-cm Cassegrain-Nasmyth telescope of the Lund Observatory, we have obtained photoelectric *UBV* observations and a number of intermediate-dispersion spectrograms covering the time of maximum and the following week. A preliminary light curve is shown in Fig. 1. Our data have been supplemented with those of other observers. The light curve is smooth during maximum and early decline, but significant fluctuations appear during the transition stage; for example, a local maximum seems clearly established on September 7.88 UT.

For ordinary novae there is a statistical relation between absolute magnitude at maximum, M_0 , and the time for a brightness decrease of three magnitudes from maximum, t_3 . McLaughlin gives¹

$$M_0 = -11.5 + 2.5 \log t_3$$

with t_3 expressed in d. For Nova Cygni we find $t_3 = 3.5$ d and $M_0 = -10.1$. The fastest non-recurrent nova hitherto known, CP Pup 1942, had $t_3 = 7$ d. For most novae, t_3 ranges from 10-200 d. Application of the above formula therefore depends on a somewhat hazardous extrapolation. It suggests, however, an unusually high luminosity. It should be noted that supernovae have values of t_3 around 50 d and absolute magnitudes from -15 to -20.

Nova Cygni is situated almost exactly in the galactic plane ($l'' = 89.8^\circ$, $b'' = -0.1^\circ$). The extinction in this direction is moderate. Assuming² an extinction of 2.5 mag for a distance modulus of 12, the nova would be ~ 800 pc away.

The photoelectric colours $U - B$ and $B - V$ indicated at premaximum a strong excess of ultraviolet and visual light

compared with the blue region, approaching the colours expected for black-body radiation. At maximum brightness the colours were $U - B = -0.10$ and $B - V = 0.65$, decreasing gradually to -0.60 and 0.20 respectively 8 d after maximum. At this stage the colours mainly reflect the relative intensities of the emission lines.

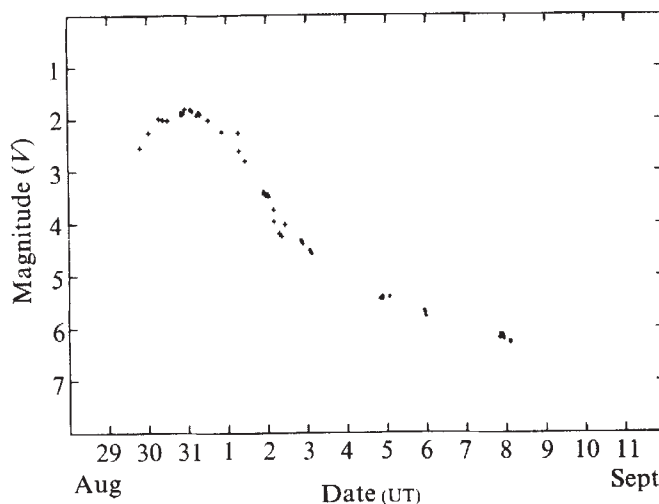


Fig. 1 Light curve of Nova Cygni 1975. Dots, our data; crosses, data from ref. 3.

The spectral changes from one day before to five days after maximum are drastic. This is demonstrated in Fig. 2, which contains intensity registrations in the blue wavelength region. The evolution is roughly that of the typical nova spectrum, though it is unusually rapid and the narrow absorption lines normally seen seem to be missing. The premaximum spectrum is dominated by very broad, but not very strong, absorption lines of for instance H, Ca II, and Fe II, displaced by 1,000 to 2,000 km s⁻¹ towards shorter wavelengths. At maximum, bright lines of the same elements appear close to their normal

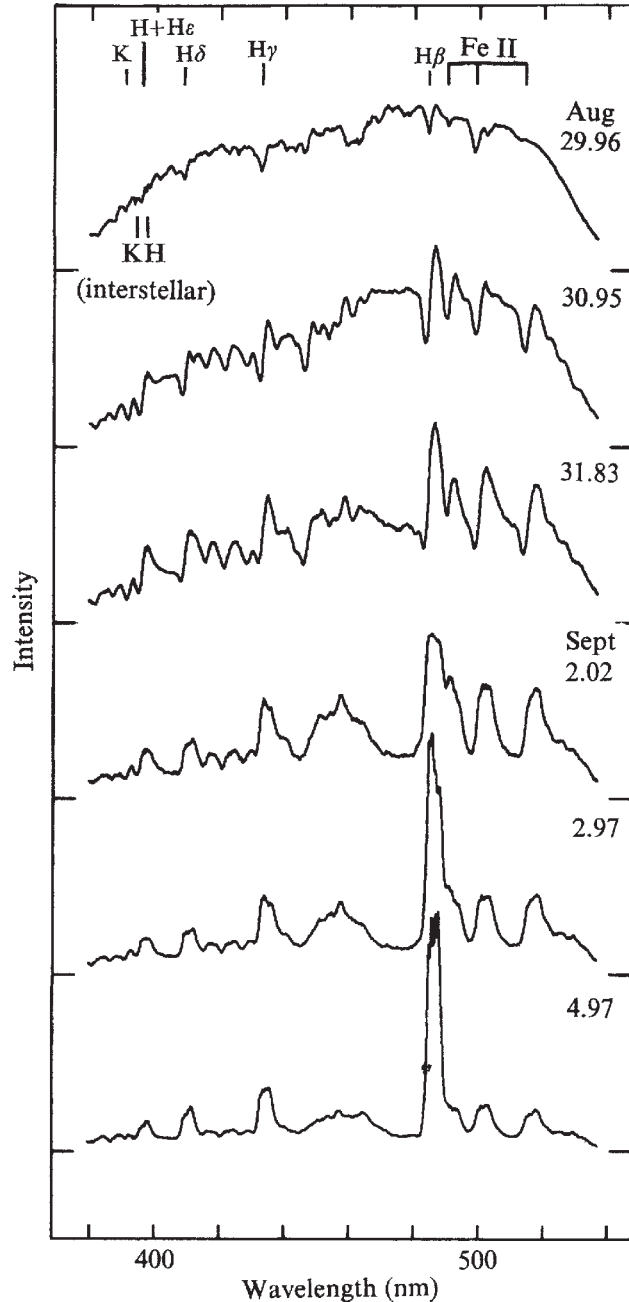


Fig. 2 Spectra of Nova Cygni. The zero-intensity point of each spectrum is indicated. Original dispersion was $83 \text{ \AA mm}^{-1}$ on IIIa-J plates.

positions. From then on the continuum weakens relative to the lines. A week after maximum most of the light comes from very broad, multiple-component emission lines. The structure of the lines changed markedly from night to night indicating that several clouds were ejected at velocities of about $1,500 \text{ km s}^{-1}$.

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The periodic transient X-ray sources

THE transient X-ray sources Ariel 1118–61 and Ariel 0535+26 have periods of 405 s (ref. 1) and 114 s (ref. 2) respectively. Rappaport and McClintock³ reported the discovery of a 283-s period in the X-ray binary 3U0900–40 (a 'continuous' source) which is known to have an orbital period $P_{\text{orb}} = 8.95 \text{ d}$. This raises the possibility that the periodic transients may be a particular stage of evolution of the more familiar class of X-ray binary of the 3U0900–40 variety. We propose that the transient phenomenon occurs during the 'sleeping phase'⁴ of the binary system when the early type star is still essentially on the main sequence, significantly underfilling its Roche lobe.

We assume that neutron stars are born rotating rapidly. Illarionov and Sunyaev⁵ have shown that a magnetic, rotating neutron star will spin down in a stellar wind by a combination of the pulsar emission and 'propeller' mechanisms to an equilibrium period P_{eq} at which the Alfvén radius is equal to the corotation radius. In general, significant accretion can occur only when $P \geq P_{\text{eq}}$ where⁵

$$P_{\text{eq}} \approx 30 \left[\frac{B_0}{10^{12} \text{ gauss}} \right]^{6/7} \left[\frac{1.5 \times 10^{-11} \dot{M}_{\odot} \text{ yr}^{-1}}{\dot{M}_A} \right]^{3/7} \left[\frac{M_{\odot}}{M} \right]^{5/7} \text{ s} \quad (1)$$

Here B_0 is the strength of the surface magnetic field, M is the mass of the neutron star, and $\dot{M}_A$ is the rate of accretion. For a neutron star accreting in a spherically symmetric wind of strength $\dot{M}_w$ we also have

$$\frac{\dot{M}_A}{\dot{M}_w} \sim 10^{-4} \left[\frac{M}{M_{\odot}} \right]^2 \left[\frac{20R_{\odot}}{a} \right]^2 \left[\frac{10^3 \text{ km s}^{-1}}{V} \right]^4 \quad (2)$$

where V is the velocity of the wind at the accretion radius and a is the binary separation. When accretion occurs the X-ray luminosity L_X is given by

$$L_X \sim 0.1 \dot{M}_A c^2 \approx 10^{35} \left[\frac{\dot{M}_A}{1.5 \times 10^{-11} M_{\odot} \text{ yr}^{-1}} \right] \text{ erg s}^{-1} \quad (3)$$

For 3U0900–40, the mean X-ray luminosity observed at present ($\sim 10^{37} \text{ erg s}^{-1}$, assuming a distance of $\sim 2 \text{ kpc}$) indicates $\dot{M}_A \sim 10^{-9} M_{\odot} \text{ yr}^{-1}$, which implies $P_{\text{eq}} \sim 4\text{--}30 \text{ s}$ for $B_0 \sim 10^{12}\text{--}10^{13} \text{ gauss}$. For $V \sim 500\text{--}1,000 \text{ km s}^{-1}$ the corresponding mass loss rate $\dot{M}_w \sim 10^{-5}\text{--}10^{-6} M_{\odot} \text{ yr}^{-1}$. Unless we adopt what may be an unacceptably high value for B_0 ($\sim 10^{14} \text{ gauss}$), the 283-s periodicity in 3U0900–40, if interpreted as a rotation period of a neutron star, indicates that spin down has occurred in a weaker wind than is currently observed.

The time scales for spin down in the pulsar (t_{CR}) and propeller (t_A) phases have been discussed^{5,6}. Assuming $B_0 \sim 10^{11} \text{ gauss}$, $\dot{M}_A \sim 10^{-11} M_{\odot} \text{ yr}^{-1}$ (equivalent to $\dot{M}_w \sim 10^{-7} M_{\odot} \text{ yr}^{-1}$) Illarionov and Sunyaev⁵ find that $t_{\text{CR}} \sim 10^7 \text{ yr}$ and $t_A \sim 10^8 \text{ yr}$. They conclude that t_A is typically greater than the evolution time, t_e , of an OB star ($t_e \sim 3.7 \times 10^6 (M_{\text{opt}}/21 M_{\odot})^{-1.9}$) (ref. 7) and that accretion is unlikely to occur during the main sequence phase.

Fabian⁶ has discussed models for Ariel 1118–61 and shows that in a weak wind ($\dot{M}_w \sim 10^{-10} M_{\odot} \text{ yr}^{-1}$) and with $B_0 \sim 10^{12} \text{ gauss}$, the spin down of a neutron star to periods of about a minute could occur in a timescale $\sim 10^8 \text{ yr}$. He suggests that sporadic enhancements in the stellar wind (from $\sim 10^{-10}$ to $\sim 10^{-6} M_{\odot} \text{ yr}^{-1}$) could produce a transient source of luminosity $L_X \sim 10^{36} \text{ erg s}^{-1}$.

In the particular case of 3U0900–40, however⁸, $M_{\text{opt}} > 20 M_{\odot}$ indicating $t_e < 5 \times 10^6 \text{ yr}$. It would thus seem that spin down may be faster than previously supposed. Fabian⁶ has

drawn attention to uncertainties in the spin down estimates in the pulsar emission phase. Likewise details of spin down during the propeller phase are not well understood⁵. The estimated time scales could well be uncertain by an order of magnitude.

There is also the possibility that the stellar wind may vary with evolution (for example, observations of τ Sco (BOV) $\dot{M}_w \sim 10^{-8} M_\odot \text{ yr}^{-1}$ (ref. 9); ε Ori (BOIa) $\dot{M}_w \sim 10^{-6} M_\odot \text{ yr}^{-1}$ (ref. 10)). Using equations (9), (11) and (12) of ref. 5 with $\dot{M}_A = 10^{-13} M_\odot \text{ yr}^{-1}$ ($\dot{M}_w \sim 10^{-9} M_\odot \text{ yr}^{-1}$) in the pulsar emission phase and $\dot{M}_A = 10^{-11} M_\odot \text{ yr}^{-1}$ ($\dot{M}_w \sim 10^{-7} M_\odot \text{ yr}^{-1}$) in the propeller phase, we find for $B_0 \sim 10^{12}$ – 10^{13} gauss, $t_{CR} \sim 10^7$ – 10^6 yr, $t_A \sim 10^7$ – 3×10^6 yr and $P_{eq} \sim 30$ – 300 s. Thus a weaker wind during the pulsar phase could reduce the overall spin down time by allowing the more efficient pulsar emission mechanism to operate for longer before the propeller takes over. Because of free-free absorption in the wind it is unlikely that the pulsar would be detectable at radio energies during spin down⁵.

Based on the above considerations we propose that spin down of the neutron star to periods of about a minute occurs while the OB star is still essentially on the main sequence. Van den Heuvel⁴ has described this phase of evolution of the pre-X-ray binary system following the formation of a neutron star, as 'the sleeping phase'. We suggest that the newly born X-ray source ($P_{eq} \sim$ minutes) may be detected during this phase as a periodic transient (for example, during an outburst) or as a weak ($L_X \sim 10^{35} \text{ erg s}^{-1}$) X-ray source. The transient nature could be attributed to any one of many possibilities: (1) the tendency of the accreting neutron star to spin up in the wind and temporarily inhibit accretion. This mechanism would only be effective when the rotation period of the neutron star is near the 'equilibrium' value appropriate to the stellar wind strength (for example, Cen X-3 during the present epoch, and possibly 3U0900–40 during its main sequence phase). (2) The Buff and McCray¹¹ thermal instability which may manifest itself in a weak wind situation. (3) Matter build up near the Alfvén radius and impulsive accretion towards the end of the propeller phase. (4) Sporadic variations in the wind strength by a factor ~ 10 .

When accretion does occur the neutron star may spin up. For 3U0900–40, the estimates⁶ indicate a current spin up rate of 10^{-2} – $10^{-1} \text{ s yr}^{-1}$ for $V \sim 500$ – $1,000 \text{ km s}^{-1}$. Since the intense wind phase ($\dot{M}_w \sim 10^{-5}$ – $10^{-6} M_\odot \text{ yr}^{-1}$) lasts for $\sim 10^4$ yr (ref. 12) until Roche lobe overflow occurs, 3U0900–40 is not expected to have spun up appreciably since its main sequence phase. When Roche lobe overflow occurs the X-ray source will probably be smothered. It is interesting to note that the optical stars of the nearly continuous X-ray sources appear to be near their Roche lobes¹³.

It is not clear whether systems like Cen X-3 and 3U1700–37 which are of short orbital period with very early type stars and strong stellar winds, would follow the evolution described above. In such systems the optical star essentially reaches its Roche lobe during hydrogen core burning and the postulated weak wind phase may be short lived. (The Roche lobe radii of Cen X-3 and 3U0900–40 are $\sim 12 R_\odot$ and $\sim 35 R_\odot$ respectively.) Estimates similar to those made for 3U0900–40 indicate that Cen X-3 is unlikely to have spun down to periods $P \gtrsim 40$ s. Indeed for Cen X-3 the currently observed X-ray luminosity ($\sim 5 \times 10^{37} \text{ erg s}^{-1}$) yields $P_{eq} \sim 2$ – 15 s for $B_0 \sim 10^{12}$ – 10^{13} gauss, consistent with the observed 4.8-s period¹⁴. This could be a system in which the rotation of the neutron star has kept up with the wind evolution. By analogy 3U1700–37 may be expected to have a short period ($\lesssim 1$ min).

In the above model the optical counterparts of periodic transients are OB stars, which are relatively unevolved and underfill their Roche lobes, in orbit about the transient with periods $P_{orb} \lesssim 20$ d. Since the 'sleeping phase' lasts typically a hundred times as long as the intense stellar wind phase⁴ we may expect to see a hundred times as many transients as nearly continuous X-ray sources, of the 3U0900–40 variety. Since

the transients are expected to be intrinsically less luminous, however, they may be detectable only during a flare.

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de Sitter's theory 'melts' Europa's polar cap

DURING the favourable passage of the Earth through the plane of Jupiter's equator in 1973 many observations of the mutual phenomena of the Galilean satellites were obtained. Since then there have been numerous preliminary analyses^{1,2} arguing for the presence of a bright polar cap on Europa (J2), based essentially on photoelectric observations of a series of occultations of J2 by J1 (Io) in which increasingly larger fractions of the north polar region of the former were covered at mid-event with each successive occultation. These events—and especially the near-grazing, or polar ones—were substantially deeper than predicted. An inevitable explanation, in view of the finding of ice³ on J2 and J3, was the suggestion of a bright north polar cap for J2.

Alternatively, it may be that at mid-event the projected minimum distance, z , between the centres of the satellites in a direction essentially parallel to Jupiter's polar axis, was smaller than predicted by Sampson's theory^{4,5} (which has been in general use since 1915 to compute the ephemerides of the Galilean satellites). This explanation at first seemed less likely for several reasons. Although recent timings of eclipses in Jupiter's shadows have shown, as have the mutual events, that the theory now suffers from considerable errors of longitude, the latitude errors (that translate directly into errors, Δz , in z) were believed to be much smaller. Furthermore, preliminary analyses of a few occultations of J2 by J1 suggested that to remove the need for a bright polar cap on J2 required Δz corrections too large to be compatible with the observed durations of the occultations.

Contrary to these early findings, we can show that the 'Δz explanation' is indeed the more plausible one. We have analysed some 30 occultations and 10 eclipses, from which we have selected 10 well-observed and characteristic light curves listed in Table 1, where 'O' and 'E' in column 1 stand for 'occults' and 'eclipses', respectively. Column 2 gives the observed mid-time of an event whose amplitude, ΔV , in visual magnitude, is in column 3. We have fitted a theoretical curve to each observed one by making least-squares adjustments to the longitudes of the satellites and to the parameters, z , R_2 , and β . Here, R_2 denotes the radius of J2 (that of J1 is well

Table 1 Data from analyses of light curves

Event	Mid-time 1973 (UT)	ΔV	de Sitter-Sampson			Solution 1		Solution 2		Δ_0
			Δz_1	Δz_2	$\Delta z_1 - \Delta z_2^*$	Δz^*	Δ_1	β	Δ_2	
1O2	June 10. 38194	0.09	86	462	-376	-426	0.0034	2.88	0.0140	0.0031
1O2	June 17. 46936	0.17	101	455	-354	-415	0.0049	1.79	0.0115	0.0039
1O2	June 24. 55613	0.24	116	447	-331	-343	0.0032	1.42	0.0073	0.0060
1O2	July 22. 89868	0.43	166	409	-243	-268	0.0070	1.15	0.0079	0.0054
1O2	Aug. 16. 70257	0.53	207	375	-168	-187	0.0043	1.07	0.0051	0.0046
1E2	Aug. 16. 73448	1.53	228	399	-171	-185	0.0088	1	0.0261	0.0098
1O2	Sept. 10. 53082	0.62	253	357	-104	-116	0.0056	1.01	0.0058	0.0055
1E2	Sept. 10. 61979	1.40	284	423	-139	-82	0.0120	1	0.0161	0.0161
1O2	Sept. 28. 30912	0.55	286	378	-92	-112	0.0043	0.96	0.0050	0.0050
2O1	Dec. 21. 02251	0.15	-124	-539	+415	+425	0.0076	1.85	0.0090	0.0056

*A positive Δz or $\Delta z_1 - \Delta z_2$ implies an increase in the projected centre-centre distance z if J1 is north of J2 (first 7 events), but a decrease if J1 is south of J2 (last 3 events).

known from its occultation of β Sco⁶) and β is, for the occulted satellite, the ratio of the average surface brightness of the occulted portion at mid-event to the mean over the entire disk. A consideration of all the total and near-total occultations and eclipses of J2 enabled us to obtain $R_2 = 1,533$ km, a downward revision of only 17 km from the micrometric value⁷. Table 1 shows the results of two solutions to the observed curves, both of which use $R_2 = 1,533$ km. The first, or 'de Sitter solution' in columns 7 and 8, sets $\beta = 1$ and obtains Δz ; the second, or 'polar cap solution' (columns 9 and 10) imposes $\Delta z = 0$ and yields β . (The longitude corrections are identical for both solutions, but are not relevant here. We also omit a third, simultaneous solution for Δz and β , resulting in β values not much different from 1.) Also shown in Table 1 are the r.m.s. residuals, Δ_1 and Δ_2 , for the two solutions, where the intensity scale was normalised to unity outside of the events. A third set of residuals, Δ_0 , was obtained independently of

Perhaps the strongest evidence for the reality of the Δz corrections comes from an independent source: de Sitter's theory^{9,10} for the Galilean satellites. Table 2 lists the proper inclinations, γ_1 and γ_2 , on a plane essentially coincident with Jupiter's equator; the proper nodes, θ_1 and θ_2 ; and the mean longitudes, l_1 and l_2 , as obtained by Sampson and by de Sitter for J1 and J2. Whereas the mean longitudes and inclinations generally agree, the nodes differ markedly, especially when brought forward to 1973. A strong case exists for favouring de Sitter's values because his reliance on heliometer and photographic measurements—in addition to eclipse observations—provided a better determination of the orbital planes of J1 to J4.

The small differences in the two determinations of γ_1 and γ_2 yield latitude discrepancies of no more than 33 km for J1 and 15 km for J2. The marked differences, however, in the positions and motions of θ_1 and θ_2 between the de Sitter (D)

Table 2 Elements for J1 and J2 (t (d) from 1900 Jan. 0.5 ET)

Author	γ_1	θ_1	l_1
Sampson	0.02725°	33.299° - 0.134068° t	142.59987° + 203.488954208° t
de Sitter	0.0317°	64.7° - 0.13214° t	142.590° + 203.48895460° t
	γ_2	θ_2	l_2
Sampson	0.46805°	290.54986° - 0.0327375° t	99.55081° + 101.374723445° t
de Sitter	0.4668°	292.81° - 0.032599° t	99.530° + 101.37472360° t

any of a model light-curve by folding the two branches of each light curve. Since these curves give no indication of asymmetry, Δ_0 provide a measure of the noise levels.

Those residuals, especially for the shallow occultations, clearly favour solution 1. The β for these shallow events correspond, when one recalls that the geometric albedo of J2 lies near 0.6 (ref. 8), to an unrealistically high surface brightness for the north polar region of the satellite. Occultations 2 and 3 are the ones that were used qualitatively to support the polar cap¹. In the case of the final entry of Table 1, the north pole of J1 was covered by J2. This event requires $\beta = 1.85$ if Δz is forced to be zero, and yet photography from Pioneer 10 has shown that this region of J1 is slightly darker than the average surface material.

Table 1 also includes two eclipses, for which Δz agrees with that of the occultations which occurred just beforehand. While the radius R_2 , derived from the occultations, changed little with Δz , R_2 obtained from the eclipses gave the discordant values 1,345 and 1,610 km when Δz was forced to be zero. When Δz was included in the solution, these values became 1,543 and 1,519 km. (For eclipses, β was set equal to unity. To have set β free would have involved a substantial, and apparently unnecessary, complication because penumbral shadowing must be considered.)

and Sampson (S) values have a much larger influence. The effect of the differing values for the inclinations and the arguments of latitude, corrected for light travel time to Jupiter, on Δz can be calculated at the mid-times of the events from Table 2 and the formula

$$\Delta z_i = a_i [\gamma_{i,D} \sin(l_i - \theta_{i,D}) - \gamma_{i,S} \sin(l_i - \theta_{i,S})], \quad i = 1, 2$$

where a_i is the semimajor axis of satellite i . The differences, $\Delta z_1 - \Delta z_2$ (km), in Table 1 agree very well with the Δz corrections derived from the light curves. There is some indication that $|\Delta z| > |\Delta z_1 - \Delta z_2|$, particularly for the shallow, 1O2 cases. In view of photometric uncertainties, we regard this as of marginal significance. If real, it would mean a slight revision of de Sitter's orbital elements and/or a minute polar brightening. The general agreement (shown in Table 1) seems to lead to a ready explanation for the behaviour of the J1-J2 events in 1973 and to add confirmation to de Sitter's theory. We are now completing the theory so that calculations of ephemerides can be made.

Whether any information on the brightness of satellite features can be obtained from these observations has yet to be shown.

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Late Cainozoic explosive eruptions in the Aleutian and Kuril Island Arcs

THE rate at which major explosive eruptions have occurred in the Aleutian and Kuril Island Arcs is recorded in tephra layers recovered in cores of the Deep Sea Drilling Project (DSDP). Major tephra eruptions occurring with the frequency and intensity currently observed in the Aleutian and Kuril Arcs may have marked effects on world climate, especially through the production of dust veils and the consequent variation in the volume of ice in the polar seas and in general atmospheric circulation¹.

Since 1760 the 36 active volcanoes in the Aleutian Island Arc have erupted 154 times². Of these, 32 were 'major explosive eruptions'². On the Kamchatka Peninsula, 23 active volcanoes of the Kuril Island Arc have erupted at

Fig. 1 Sedimentation rate against sub-bottom depth curves for DSDP sites 178 (a), 183 (b) and 192 (c) in the northern Pacific ocean. Palaeontological data from refs 9 and 10.

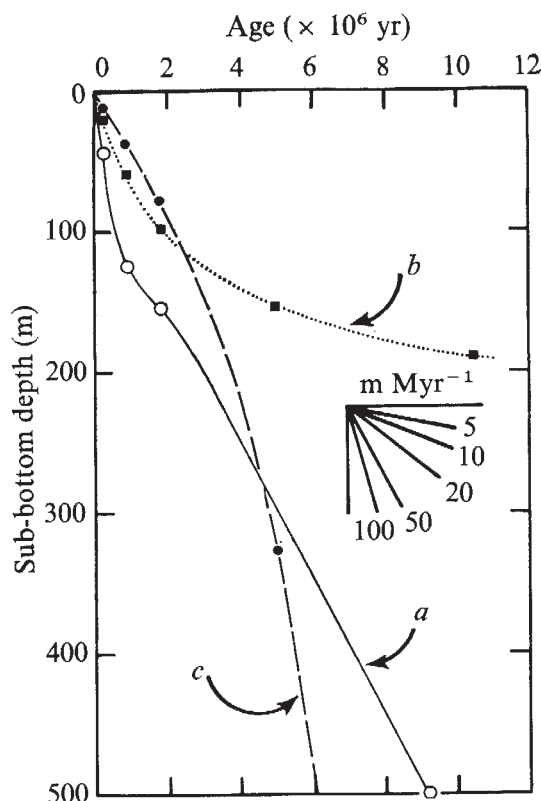
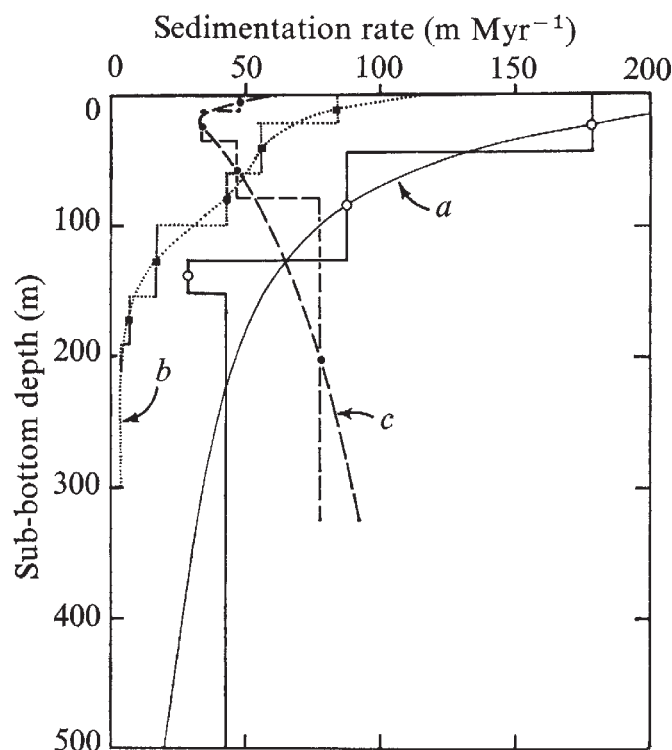


Fig. 2 Age against sub-bottom depth curves for DSDP sites 178 (a), 183 (b) and 192 (c). Data and age assignments as in Fig. 1.

least 137 times since 1729 (ref. 3). Several of these eruptions were sufficiently energetic and voluminous to blast tephra into the stratosphere and to blanket extensive areas with pyroclastic debris. The 1912 eruption of Mount Katmai in Alaska deposited 25 cm of ash 160 km to the south-east, at Kodiak Village⁴, and 7 cm of ash fell 560 km from the mountain⁵. The 1956 eruption of Bezymianny Volcano on Kamchatka produced an ashcloud 45 km high and deposited ash over 40,000 sq km (ref. 6). The 1964 blast at Shiveluch Volcano on Kamchatka deposited 2.5 mm of ash on the Komandorsky Islands, 400 km to the east⁷. The violent eruption of Mount Spurr, Alaska in 1953 shot a mushroom cloud upwards to a height of 21 km in about 40 min (ref. 8).

DSDP sites 178 and 183 are in the Gulf of Alaska, 450 and 250 km, respectively, south-east of the Aleutian volcanic chain^{9,10}. Site 192 lies 350 km east of the nearest volcanoes in the Kuril-Kamchatka chain¹⁰. All three lie downwind from active volcanic centres. Cores recovered at the sites contain large quantities of volcanic ash, particularly in the Pleistocene sections.

The rate of ash fall at each site has been estimated by using each recovered interval as a sample of the rate at which tephra was deposited at each site. The time span represented by each recovered interval has been estimated by dividing the sedimentation rate by the length of the recovered interval. The sedimentation rate was obtained from an enlargement of the sedimentation rate against the sub-bottom depth curve (Fig. 1). The number of ash layers occurring within the time span represented by each recovered interval has been normalised to an arbitrary deposition rate of tephra layers each 1 Myr. The absolute age for the midpoint of each recovered interval has been estimated from the sub-bottom depth against the age curve (Fig. 2) and plotted against the rate of tephra arrival (Fig. 3).

This technique of using each recovered interval as a 'sample' of the rate of incoming tephra is required because core recovery is very low. At site 178 only 39% of the tephra-rich interval was recovered, at site 183 only 20%,

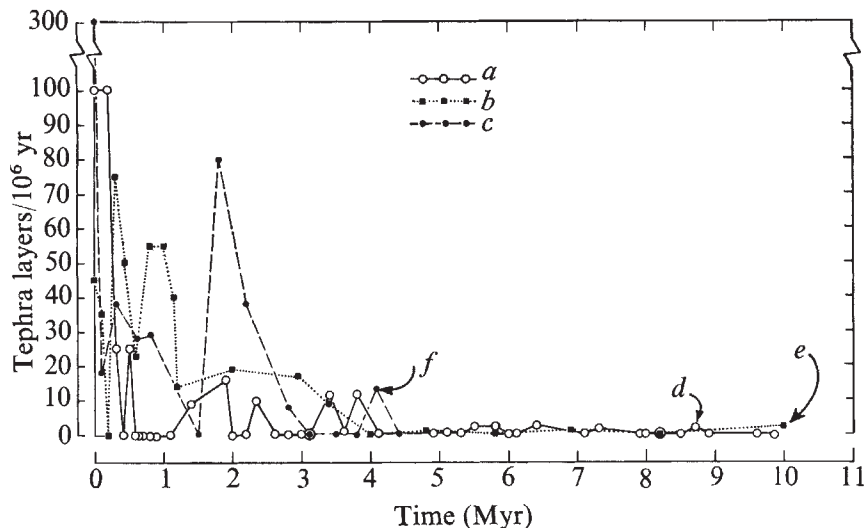


Fig. 3 Rate of ashfall in the northern Pacific ocean at DSDP sites 178 (a), 183 (b) and 192 (c). d, Oldest ash, site 178; e, oldest ash, site 183; f, oldest ash, site 192.

and at site 192 only 30%. Therefore, the actual number of ash layers is rather insignificant, and the rate of ash accumulation is a more appropriate parameter for analysis.

Tephra erupted in quantities sufficient to form distinct layers apparently began falling in the Gulf of Alaska about 10 Myr ago—but at a rate which averaged less than 1 layer every Myr—until about 4 Myr ago. Both curves from the Gulf of Alaska sites show a sporadic increase of up to 20 layers Myr^{-1} between 4 Myr and about 1.2 Myr BP, a decrease to 0 at 0.2–0.4 Myr BP, and a dramatic increase to rates of about 100 layers each Myr at present.

In the north-western Pacific, ash does not appear as discrete layers in the cores until about 4 Myr BP. It appears again at about 1.8 and 0.3 Myr BP, with an increase to 300 layers Myr^{-1} at present.

Summing up the data for sites 178 and 192 suggests that tephra layers are accumulating in the far northern Pacific at a rate of about 1 layer every 2,500 yr. This rate in itself is probably not indicative of any major influence on the Earth's atmosphere, for volcanic outbursts and associated effects are of short duration¹. But because the layers in the cores are usually several centimetres thick and accumulated hundreds of kilometres from possible sources, the data probably only reflect major cataclysmic eruptions, and may only indicate a quickening pace or tempo of volcanism.

A survey of explosive volcanism over the past two centuries indicates there have been two primary latitudinal source belts of volcanic ejecta: a narrow belt just below the Arctic Circle, and a broad belt centred on the Equator¹¹. In the Arctic Circle belt the height of the tropopause is only two-thirds of that over the Equator. A less explosive event in the northern latitudes could contribute as much or more debris to the northern stratosphere as an eruption on the Equator of the magnitude of Agung¹¹.

Data on the number of volcanic ash layers sampled at many DSDP sites indicate an apparent global increase in Quaternary explosive volcanism¹². A comparison of the ashfall rate curve for the northern Pacific sites with volcanic debris accumulation rates from the western Pacific¹³, the Caribbean Sea¹⁴, and other volcanic regions¹², suggests that most island arcs are experiencing comparable rates of cataclysmic eruption. The total world rate may possibly be 5–10 times that in the northern Pacific, which would suggest a major eruption every 250–500 yr. Ash layers preserved in the Antarctic ice sheet suggest even higher figures with infalls during the peak period of volcanic activity (20,000–16,000 yr BP) reaching 30–40 a century¹⁵. Those rates, coupled with the apparently prodigious explosive eruptive history not recorded in the deep sea, could certainly have lasting and significant effects on the albedo and air temperature of the Earth.

The magnitude of any effect is difficult to estimate directly, but it is known that dust injected into the stratosphere at high latitudes seems to tend to spread as a veil between the pole and about 30° latitude in either hemisphere¹. Volcanic dust may cause an overall decrease in the amount of solar radiation received and change the meridional gradient of the insolation, and thus the north-south temperature pattern which governs the speed and pattern of the general circulation¹. Depletion of the incoming solar beam is greatest at high latitudes, and depression of surface temperatures is probably also greatest at high latitudes¹. The 1912 Katmai eruption was followed by a 10% increase in the total extent of sea ice at the end of the melting season—an effect which lingered through seven successive summers¹⁶. On a longer time scale there does seem to be some evidence that the late Wisconsin and Neoglacial ice advances were contemporaneous with major phases of volcanic activity^{15,17} and the greatly increased worldwide level of volcanism¹² closely coincides with the major development of the late Cainozoic glacial ages.

A complicating factor affecting these calculations is the north-westward motion of the Pacific Plate relative to the North American and Asian Plates. Using a figure of 6×10^{-7} degrees yr^{-1} for the rate of motion between those plates, Ness and Kulm¹⁸ calculated about 220 km of north-westward motion for site 178 during the past 5 Myr. Similar movements probably affected sites 183 and 192, for all the sites lie on the same plate. As a result of this motion, at least some of the increased rate of ashfall may be attributable to the DSDP sites in the northern Pacific simply moving nearer the volcanic sources. This may explain why evidence is not seen in the cores for the middle Miocene episode of calc-alkaline magmatism reported from the Aleutian Island Arc¹⁹.

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Antarctic glaciation and early Tertiary vegetation

PALYNOLOGICAL evidence obtained from drilling in the Ross Sea by the Glomar Challenger suggests that vegetation persisted in the area until the late Oligocene and results from the same drilling programme also suggest that ice-rafting of clastic debris commenced in the late Oligocene¹. The vegetation reflected in the pollen spectrum therefore seems to have been contemporaneous with early phases of glaciation.

Pollen and spores were recovered from site 270, which was drilled off the edge of the present Ross Ice Shelf (Fig. 1). The sequence at site 270 is shown in Fig. 2. Sediments of probable preglacial origin at the site are thin, consisting of a unit of quartz sand 1 m thick, lying on basal breccias and gneisses, and a further metre of glauconitic sandstone. Overlying these is a thick, pebbly, silty claystone of glacial marine origin. Subunits can be identified within this thick unit on the basis of the presence or absence of stratification. The preglacial glauconitic sandstone has been dated at 26 ± 0.4 Myr using the K-Ar method², an age with accords with that suggested by rare dinoflagellates in the unit³. The change from preglacial to glacial marine conditions occurred over a short time interval, since the lower subunits of the glacial marine sequence have also been dated as Oligocene on the basis of their contained foraminifera⁴.

Samples of preglacial and glacial marine units were macerated for pollen and spores. The preglacial units were barren of these microfossils, but all of the samples from the glacial marine unit were productive, though to a varying degree. The yield was highest in the basal subunit, 2J (Fig. 2), a laminated, burrowed siltstone with rare dropped pebbles. Degraded tissues, leaf cuticles and vascular fragments were also common in this subunit, suggesting deposi-

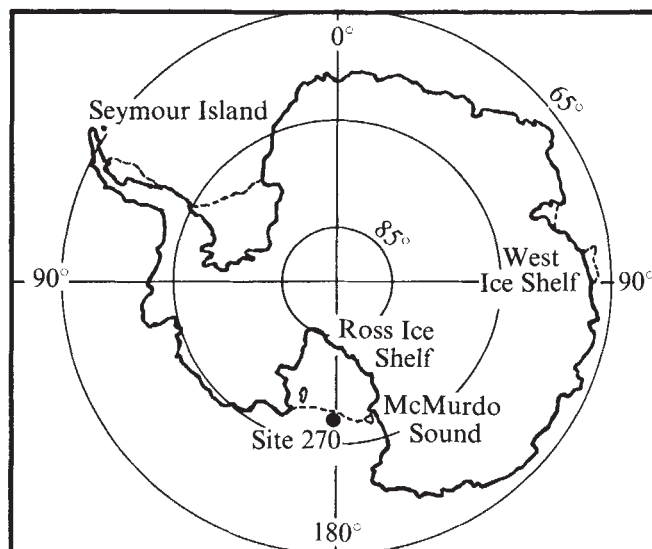


Fig. 1 Map showing Antarctic localities from which Tertiary plant microfossils have been recovered.

tion at no great distance from a vegetation source.

As in all glacial sequences, it is difficult to ascertain whether the plant microfossils in subunit 2J were derived from a parent vegetation which flourished during deposition of the unit, or whether they are recycled from older deposits. Modern glacial marine sediments on the Antarctic continental shelf contain recycled pollen and spores ranging in age from Palaeozoic to early Tertiary⁵⁻⁷. But in the assemblages from subunit 2J there are no forms which are obviously recycled; there is none derived from Palaeozoic and Mesozoic sequences in the Transantarctic Mountains, nor any of the dinoflagellate cysts which are abundant in the Black Island and Minna Bluff erratics. All of these older forms do, however, occur as a clearly recycled element in the upper parts of the glacial marine sequence at site 270. Their absence from the basal section suggests that little recycling occurred during deposition of the lower subunits, and that the pollen present reflects a parent vegetation that coexisted with the earliest glaciers.

Form species identified in subunit 2J, and their probable

Table 1 Form species of spores and pollen recovered from subunit 2J, site 270, and their probable botanical affinities

Pollen	Affinity
<i>Podocarpidites</i> spp.	Podocarpaceae
<i>Lygistipollenites florinii</i> (Cookson and Pike) Stover and Evans	Podocarpaceae (cf. <i>Dacrydium</i> spp.)
<i>Phyllocladites mawsonii</i> Cookson	Podocarpaceae (cf. <i>Dacrydium franklinii</i>)
<i>Microcachrydites antarcticus</i> Cookson	Podocarpaceae (cf. <i>Microcachrys</i>)
<i>Podosporites microsaccatus</i> (Couper) Dettman	Podocarpaceae
<i>Nothofagidites heterus</i> (Cookson) Stover and Evans	<i>Nothofagus</i> (<i>brassi</i> group)
<i>N. matauraensis</i> Couper	<i>Nothofagus</i> (<i>brassi</i> group)
<i>N. vansteenisii</i> (Cookson) Stover and Evans	<i>Nothofagus</i> (<i>brassi</i> group)
<i>N. flemingi</i> (Couper) Potonie	<i>Nothofagus</i> (<i>fusca</i> group)
<i>N. cf. asperus</i> (Cookson) Stover and Evans	<i>Nothofagus</i> (<i>menziesii</i> group)
<i>Tricolpites</i> cf. <i>matauraensis</i> Couper	Unknown
<i>T. cf. fissilis</i> Couper	Unknown
<i>Triporopollenites</i> sp.	Unknown
<i>Proteacidites minimus</i> Couper	Proteaceae
<i>P. subscabratus</i> Couper	Proteaceae
<i>P. cf. pseudomoides</i> Stover	Proteaceae
<i>P. spp.</i>	Proteaceae
<i>Myrtaceidites</i> spp.	Myrtaceae
Spores	
<i>Cyathidites</i> spp.	Cyatheaceae
<i>Gleicheniidites cerciniidites</i> (Cookson) Dettman	Gleicheniaceae
<i>Laevigatosporites major</i> (Cookson) Krutzsch	Unknown
<i>Polypodiisporites</i> sp.	Polypodiaceae
<i>Lycopodiumsporites</i> sp.	Lycopodiaceae
<i>Stereisporites antiquasporites</i> (Wilson and Webster) Dettman	Sphagnaceae

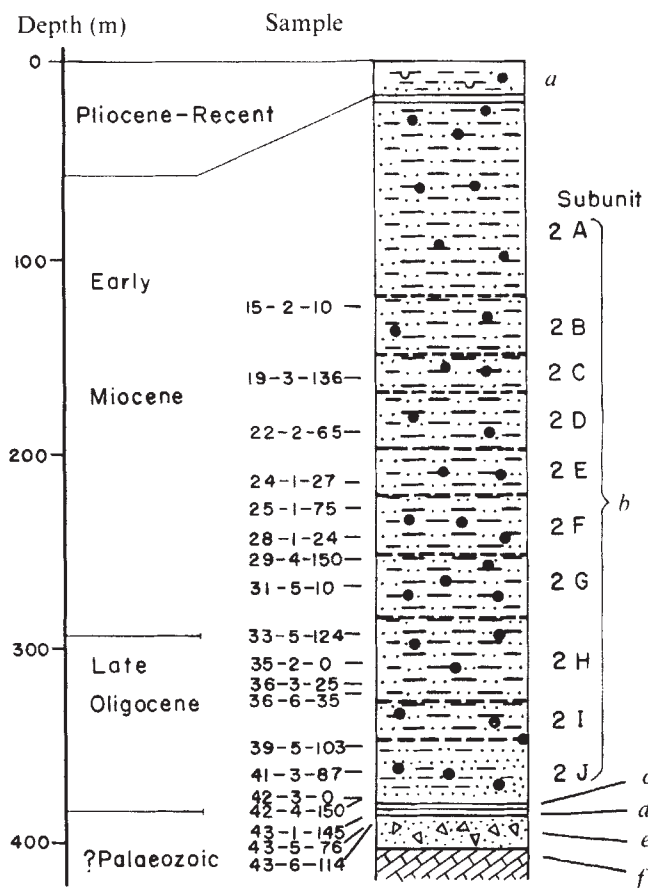


Fig. 2 Lithological sequence at site 270. Lithological subdivisions are shown to the right of the column; positions of cores from which palynological residues have been recovered are shown on the left. *a*, Unit 1: diatomaceous silty clay with pebbles; *b*, unit 2: silty claystone with pebbles (glacial marine); *c*, unit 3: greensand; *d*, unit 4: quartz sand; *e*, unit 5: breccia; *f*, calc-silicate gneiss.

botanical affinities, are listed in Table 1. Numerically, the assemblages are dominated by pollens of the *Nothofagus* type, with fossil forms similar to pollen of the extant *fusca* group present in slightly greater abundance than those of the *brassi* group. Pollen of Proteaceae is also well represented, but not diverse, with small, smooth walled pollens referable to *Proteacidites minimus* being most common. Small myrtaceous pollens, not identifiable below family level, occur rarely, as do tricolpate and triporate pollens that could be derived from a variety of parent families. Among gymnosperm pollens, podocarpaceous types predominate: the long ranging *Microcachrydites antarcticus* and *Podosporites microsaccatus* are most common. *Lygistipollenites florinii*, of mid-Palaeocene to Miocene range in south-eastern Australia⁸, is a rare component. Cycadophyte pollens were not identified. Fern spores are rare, and chiefly suggest Cyathaceae, but include rare polyodiacean types.

The assemblage is much less diverse than those of early Tertiary sequences in Australia and New Zealand. It is also less diverse than the Seymour Island fossil assemblages. It is almost identical with that from the Black Island erratics, which on dinoflagellate evidence, is probably late Eocene. The microflora from the erratics has been interpreted as reflecting Southern Beech forest of the kind at present growing in cool temperate South America and New Zealand⁹. Temperatures cooler than those of Eocene New Zealand are, however, suggested, as pollen types indicative of warmth in the New Zealand Eocene are absent.

The data presented here reflect a late Oligocene parent vegetation in the Ross Sea region which was essentially

similar to that of the erratics. A *Nothofagus*-dominated vegetation, with subordinate podocarps, Proteaceae and Myrtaceae, seems to be represented by the pollen spectrum. The available record suggests that little change in floristic composition occurred with the increasing deterioration of the Antarctic climate from the Eocene through to the Oligocene. There is no evidence of a vegetation phase dominated by herbaceous elements; taxa which are important in the modern sub-Antarctic vegetation, such as Graminae, Cyperaceae, Compositae and Umbelliferae have not been identified. It may be that those groups, which, on a global basis, achieved their major radiation only in the Neogene, were prevented from becoming established in the Ross Sea region by the onset of conditions limiting growth there early in that interval. Alternatively, this absence may only reflect the inadequacies of the fossil record.

The relationship between vegetation cover and the extent of glaciation in Antarctica in the early Cainozoic remains problematical. The persistence of some form of vegetation around the Ross Sea until at least the late Oligocene is not at odds with geological evidence bearing on the earliest stages of glaciation. Sedimentation rates have been used in this context to give a broad guide to the extent of ice cover¹⁰. High rates of sedimentation are believed to reflect an environment with wet base, 'temperate' glaciers calving bergs into a relatively open sea; by contrast, periods of intense cold, with the major development of ice shelves are distinguished by slow sedimentation and erosion by currents beneath the ice. High sedimentation rates (41 m Myr⁻¹) prevailed through the Oligocene and early Miocene sequence at site 270¹¹. This, and the observation that pebbles in this part of the sequence are derived from inland Marie Byrd Land, suggest that the Ross Ice Shelf was not in existence during that interval, and that glaciers from Marie Byrd Land calved directly into an open Ross Sea embayment. Such a situation seems compatible with limited vegetation cover, which was perhaps confined to coastal zones and interglacial elevations.

It is not possible to determine precisely when adverse environmental conditions eliminated the Ross Sea vegetation entirely. The Miocene part of the drilled sequences in the region yields pollen similar in composition to that of the Oligocene and Eocene, but this occurs in very low frequencies only, and there is evidence of extensive reworking from older deposits. It seems likely, however, that the almost complete elimination of vegetation occurred sometime during the Miocene; this event probably occurred appreciably before the major ice advances of the early Pliocene¹.

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The fault pattern of a slow-spreading ridge near a fracture zone

IN July, 1973 a 30,000 km² area of the median valley and crest of the Mid-Atlantic Ridge near 37°N (Fig. 1) was surveyed with the long range side-scan sonar, GLORIA¹. A mosaic of overlapping sonographs was produced, part of which is shown in Fig. 2, representing the view of the sea floor which would be obtained if the water were removed and the sea bed illuminated from the north-west at a Sun elevation of about 15°. On the mosaic, white areas represent relatively strong backscattering targets, and the black areas are either in shadow or are weakly backscattering sediment ponds.

On the mosaic, numerous sub-parallel linear echoes, oriented at about 020°, and therefore parallel to the median valley, can be followed continuously for tens of kilometres. Narrow beam echo-sounder profiles, roughly at right angles to GLORIA's track, have established that these echoes come from steep slopes on the sea floor (ref. 2, and R. B. Whitmarsh and A. S. Laughton, unpublished). Many of these echoes have a gentle curvature to the right as they approach fracture zone valleys, as illustrated in the north-eastern quadrant of Fig. 2. Other echoes have a more NE-SW trend and are oriented at less than 50° to the fracture zone valley. These echoes cut across the 020° lineations, giving the impression of an abrupt change in direction which often approximately coincides with the fracture zone escarpment, as can be seen in the south-eastern quadrant of Fig. 2.

The first order relief in the crestal zone of a slow-spreading ridge is tectonically controlled³⁻⁵. Volcanic processes give rise to only relatively short ($\lesssim 5$ km)⁶ and relatively low linear ridges and occasional isolated shield mountains. The above evidence, plus the fact that many echoes are attributable to local steepening of slopes rather than to ridges, suggests that most linear echoes on the mosaic are attributable to fault scarps. Furthermore, near-bottom observations within the median valley have de-

Fig. 1 Bathymetry of the Mid-Atlantic Ridge near 37°N (FAMOUS area), contour interval 100 fathoms, depths < 1,000 fathoms are stippled (courtesy J. D. Phillips¹⁶). Box is area of Fig. 2.

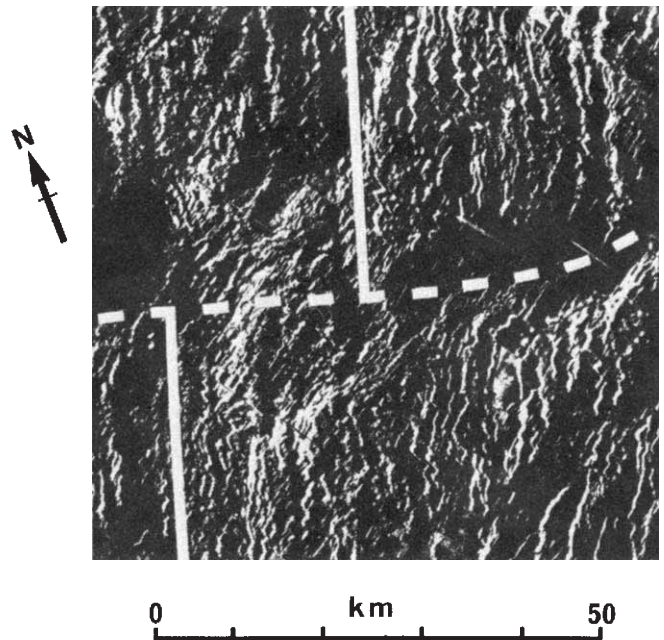
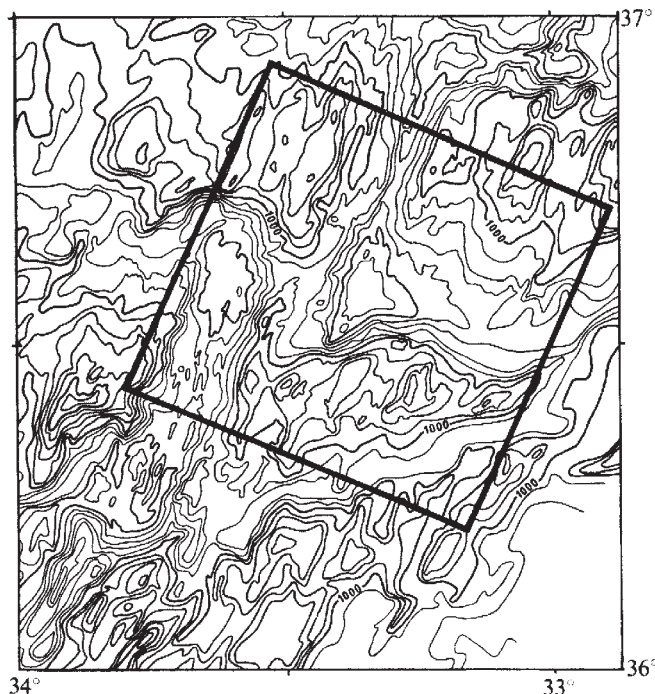


Fig. 2 Part of the GLORIA sonograph mosaic from the box in Fig. 1. The median valley and fracture zone B axes are indicated by continuous and dashed lines respectively. The sinuous nature of some targets is an artefact from the yaw of the sonar vehicle.

monstrated that scarps of large normal faults are exposed there⁷. Thus we may use the echoes on the mosaic as a basis for discussing the tectonics of a slow spreading ridge crest. At the same time an explanation is sought for (1) the gently curved fault outcrops and (2) the set of faults which trend obliquely to them.

Figure 3a is a schematic representation of the fault trends (echoes) in the area of Fig. 2. In the mosaic the fault scarps lying east of the median valley are better delineated because they face predominantly inwards, and thus towards GLORIA; those lying west of the axis are rarely insonified. For this discussion, however, it is assumed that there is rotational symmetry between east and west, as shown in Fig. 3a.

The gently curved and oblique faults cannot be explained by drag across the transform fault section of the fracture zone because the sense of drag is wrong. Nor can the gentle curvature in the NE and SW quadrants of Fig. 3a be attributed to the intersection of fault planes, striking parallel to the median valley and dipping towards its centre, with the cross-cutting valley of the fracture zone, since, even if faults are proposed which have had much less than are actually observed⁷, it is not possible to obtain a fault trace of approximately the correct curvature, given the bathymetry of fracture zone B, nor is this model consistent with the morphology in the other two quadrants (Fig. 3b).

If the fault surface is no longer constrained to be a plane, but the strike lines are still straight and parallel to the median valley, a non-planar subterranean fault surface can be derived from the fault trace on the sea bed and the seabed contours. The resulting surface is non-cylindrical and has a near vertical dip on high ground but reduces rapidly to about 10° at the level of the floor of the transform fault valley. Even if a cylindrical surface were forced through our observations the horizontal motion towards the median valley at the foot of a fault block would create difficult geometrical problems in a quasisteady-state sea-floor spreading model.

We prefer to explain the gently curved fault traces as resulting from steeply dipping faults resulting from caldera subsidence at the centre of the median valley. This hypo-

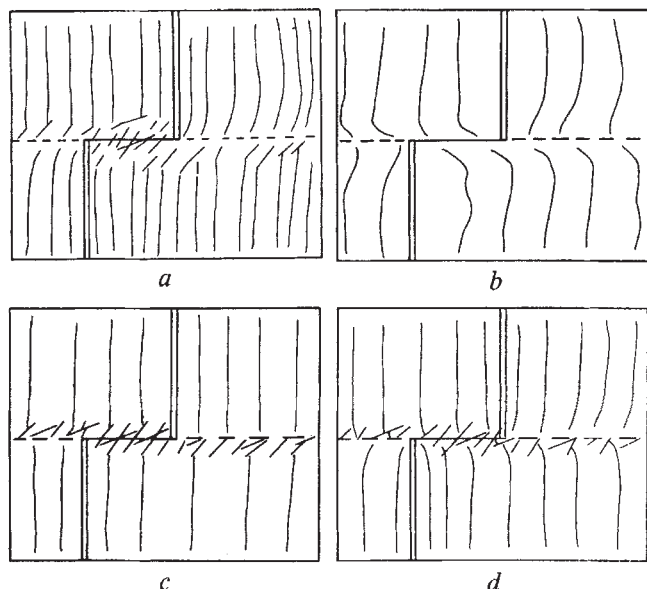


Fig. 3 a, Schematic sketch of fault trends observed in Fig. 2. b, Fault pattern due to a set of inward dipping low angle faults striking parallel to the median valley. c, Fault pattern which would be associated with wrench faulting along the transform fault together with vertical normal faults striking parallel to the median valley. d, Fault pattern produced by a combination of the processes of caldera subsidence and wrench faulting (see text).

thesis differs fundamentally from the previous ones in that the faults are not assumed to have had an initial strike parallel to the median valley axis along their whole length. The curvature reflects the shape of the underlying magma chamber at the time of collapse.

An analogy is drawn here with the elliptical caldera faults common within central vent volcanoes⁸ and with spindle-shaped graben (*fuseau allongé*) described in Afar⁹. Intermittent caldera subsidence has recently been invoked to explain features of the natural seismicity in the median valley¹⁰. The shape of the caldera faults can be explained if there is a cigar-shaped magma chamber beneath and parallel to each median valley segment. When the magma chamber pressure falls, the overlying lavas subside along near vertical fault planes, curved in plan, which outline the caldera. The axial intrusion zone, however, in which volcanic extrusives accumulate at the seafloor, remains a strip of uniform width between the caldera faults so that, for example, magnetic reversal boundaries remain straight.

The caldera subsidence model requires that, near fracture zones, faults should curve towards the median valley in all four quadrants in Fig. 3a. This is not seen on the mosaic in the NW and SE quadrants possibly because the trends would be nearly normal to GLORIA's track. It can be detected, however, in part of the NW and SE quadrants on a very detailed bathymetric chart of the median valley floor (ref. 16 and J. D. Phillips, unpublished charts) in the form of curved ridges and scarps.

Within the offset region between the valley axes, the caldera faults in the NW and SE quadrants seem to be disrupted and offset by the second set of faults which are oriented at $< 50^\circ$ to the fracture zone. The latter faults represent the normal and shear faults associated with wrench faulting along the transform fault. These are well known both from clay model experiments¹¹⁻¹³ and from field work on land¹⁴. Similar faults exist along a transform fault zone in central Afar¹⁴. Along wrench fault zones, normal faults are developed at $30-50^\circ$ to the main fault, and shear faults (Riedel shears) at angles $< 20^\circ$. Trends of both relative orientations can be distinguished in the sonar mosaic and were also found by Detrick *et al.*¹⁵ in a detailed sonar study near the 37°N fracture zone (Fig. 1). In a

situation of steady-state seafloor spreading these faults would give rise to the pattern in Fig. 3c. Each oblique fracture generated along the transform fault is eventually cut in half by this fault and the halves are carried away in opposite directions by seafloor spreading.

When these two tectonic processes are combined in a steady-state seafloor spreading model, the fault pattern of Fig. 3d is obtained. As the curved caldera subsidence faults in the NW and SE quadrants are carried away from the valley axis they become increasingly dissected and offset by the shear faults associated with wrench faulting, and thus are not detected there as continuous features.

A consequence of this model is that the pressure of magma in the chamber supplying the injection zone should pulsate in such a way that new caldera faults are produced at least as often as every 160,000 yr, according to the spacing between caldera faults. Uplift of the ridge crest over a width of several kilometres occurs as pressure increases and this is followed by more localised collapse along caldera faults, when pressure falls, giving rise to the median valley topography.

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Post-oxide phases of olivine and pyroxene and mineralogy of the mantle

I REPORT here experimental confirmation of the prediction¹ that oxide mixtures with either olivine or pyroxene stoichiometries are not stable mineral assemblages under lower mantle conditions. Pyroxene with a few percentages of Al_2O_3 transforms into the garnet structure at high pressure². Further I have found that garnets rich in pyrope transform to the orthorhombic perovskite phase at loading pressures above 270 kbar and temperatures above 800°C . For those reasons, and in order to allow the sample to absorb the laser beam with maximum efficiency, I used a glass form of 95% by weight $(\text{Mg}_{0.75}\text{Fe}_{0.25})\text{SiO}_3 + 5\%$ (weight) Al_2O_3 as a starting sample. This material is equivalent to a mixture with 93.7 mol % of pyroxene plus 6.3 mol % of pyrope-rich garnets, and is identical to that studied by Ringwood and Major in their synthesis of the garnet phase³.

Fine powder of the starting material was compressed in a diamond-anvil press fitted with a lever and spring assembly and was irradiated by a cw YAG laser while the sample was maintained under steady pressure. The quenched sample was removed to a modified 57.3 mm Debye-Scherrer camera for X-ray diffraction study. The details of the experimental procedure have been described elsewhere⁴.

X-ray diffraction data for the glass sample quenched from a loading pressure of about 260 kbar and temperature of about $1,500^\circ\text{C}$ are shown in Table 1. The pattern can readily be indexed as a mixture of garnet (with a lattice parameter

$a_0 = 11.502 \pm 0.013$ Å), spinel (with $a_0 = 8.113 \pm 0.017$ Å), stishovite, and the orthorhombic perovskite phase (with $a_0 = 4.799 \pm 0.008$, $b_0 = 4.959 \pm 0.008$, $c_0 = 6.905 \pm 0.011$ Å, $Z = 4$, and possible space group of Pbnm). The lattice parameter for the garnet is identical to that reported by Ringwood and Major³. I suggest that the garnet (which is not the same garnet as those of pyrope-almandite solid solutions found in nature) has disproportionated into a mixture of spinel plus stishovite; a slight amount of Al_2O_3 may be retained in the garnet or may exist as a separate phase of corundum which may not be detectable in the X-ray pattern. Spinel and stishovite (perhaps Al_2O_3 too) may further combine to form the perovskite phase.

Among the 36 observed reflections in Table 1, 30 can be indexed on the basis of an orthorhombic perovskite structure. Out of the 30, 16 are not interfered with by reflections of other observed phases. The relative intensities for strong reflections of the observed and calculated orthorhombic perovskite phase of ScAlO_3 (ref. 5) and MnVO_3 (ref. 6) are also listed in Table 1 for comparison. The general agreement is remarkable.

Could the hypothesised metastability be attributable to the fact that the perovskite phase was synthesised from a glass? In dismissing that possibility, I used an intimate mixture of olivine $(\text{Mg}_{0.75}\text{Fe}_{0.25})_2\text{SiO}_4$ with amorphous SiO_2 and Al_2O_3 in the same proportion as in the glass sample. With a few weak reflections corresponding to olivine, similar results (Table 1)

were obtained in the mixture sample under the same P, T conditions. With increasing pressure, the phase transformations observed in the $(\text{Mg}_{0.75}\text{Fe}_{0.25})\text{SiO}_3 + \text{Al}_2\text{O}_3$ system are inferred to be: garnet $\rightarrow$ spinel + stishovite + Al_2O_3 (?) $\rightarrow$ perovskite.

To examine the pyroxene system two samples with the pyroxene stoichiometry were studied at 280 kbar and 1,500 °C. One is the intimate mixture of $(\text{Mg}_{0.75}\text{Fe}_{0.25})\text{O}$ with silicic acid in the pyroxene proportion; the other is a glass form of $(\text{Mg}_{0.7}\text{Fe}_{0.3})\text{SiO}_3$. The latter is identical to that used by Ringwood and Major².

The perovskite phase has been observed in both samples. The X-ray diffraction data for the mixture sample comprise three phases (ferropericline, stishovite, and perovskite). The lattice parameters are $a_0 = 4.242 \pm 0.001$ Å for ferropericline and $a_0 = 4.793 \pm 0.004$, $b_0 = 4.937 \pm 0.004$, and $c_0 = 6.889 \pm 0.006$ Å for perovskite. The molar volume for the perovskite phase of $(\text{Mg}_{0.75}\text{Fe}_{0.25})\text{SiO}_3$ thus calculated is 24.58 ± 0.04 cm³ mol⁻¹, which is 3.7% smaller than that of its isochemical mixture $(\text{Mg}_{0.75}\text{Fe}_{0.25})\text{O} + \text{SiO}_2$ (stishovite). The latter value, 3.7%, is identical to that observed in the perovskite phase of MgSiO_3 (ref. 7).

The results obtained in the glass sample of $(\text{Mg}_{0.7}\text{Fe}_{0.3})\text{SiO}_3$ are almost identical to those for the olivine system except for the absence of the olivine phase and for strong reflections from stishovite.

Table 1 X-ray diffraction data (room temperature and 1 bar pressure) for a glass of 95% (weight) $(\text{Mg}_{0.75}\text{Fe}_{0.25})\text{SiO}_3 + 5\%$ (weight) Al_2O_3 quenched from a loading pressure of about 260 kbar and temperature of about 1,500 °C (CoK α)*

Observed		Garnet		Spinel $(\text{Mg}_{0.75}\text{Fe}_{0.25})_2\text{SiO}_4$			Stishovite SiO_2			Perovskite		
$I/I_{100}^\dagger$	$d(\text{Å})$	$d(\text{Å})$	hkl	I/I_{100}	$d(\text{Å})$	hkl	I/I_{100}	$d(\text{Å})$	hkl	$d(\text{Å})$	hkl	I/I_{100}
30	3.45									3.45	110,002	
35	3.09									3.09	111	20
50	2.96							2.96	110	100		
50	2.88	2.88	400	60	2.87	220	25					
80	2.576	2.572	420	100								
20	2.477									2.480	020	18
100	2.443				2.446	311	100			2.440	112	100
20	2.400									2.399	200	20
30	2.344	2.348	422	30	2.342	222	5			2.334	021	
40	2.259	2.256	510	30				2.25	101	18	2.266	201
10	2.198									2.203	120	
30	2.155									2.160	210	
50	2.090	2.100	521	20				2.09	200	1	2.088	013
30	2.062									2.061	211	30
30	2.011				2.028	400	50			2.014	022	
30	1.978							1.98	111	35	1.970	202
40	1.920	1.917	600							1.915	113	
45	1.863	1.866	611	40				1.87	210	13	1.857	122
40	1.737	1.734	622									
70	1.723									{ 1.726	004 }	55
5	1.690									{ 1.724	220 }	
5	1.679									1.687	023	
5	1.661	1.660	444	30						1.673	221	
<5	1.635									1.661	203	
40	1.596	1.595	640	60						1.630	014	
20	1.559				1.561	333	25			{ 1.600	300 }	
50	1.537	1.537	642	100				1.53	211	50	1.592	123
20	1.523									1.558	301	
<5	1.483									{ 1.524	131 }	20
10	1.438	1.438	800	30	1.434	440	65	1.478	220	18	{ 1.522	310 }
30	1.421									{ 1.491	032 }	
10	1.405									1.487	311	
30	1.395											
<5	1.254	1.255	842	60							1.424	132
<5	1.237				1.237	533	10	1.235	301	25	1.401	204
15	1.219							1.215	112	9	1.393	312
											1.253	322
											1.240	040
											1.220	041

*Data for garnet, spinel, stishovite and perovskite were obtained from the following lattice parameters and other relevant literature: Garnet— $a_0 = 11.502$ Å, the relative intensities (I/I_{100}) are assumed to be similar to those of pyrope-rich garnet. Spinel— $a_0 = 8.113$ Å, I/I_{100} were estimated from ref. 11. Stishovite—from ref. 12. Perovskite— $a_0 = 4.799$, $b_0 = 4.959$, and $c_0 = 6.905$ Å; I/I_{100} are those of strong reflections of orthorhombic perovskite phase of ScAlO_3 (ref. 5) and MnVO_3 (ref. 6) for comparison.

[†]Estimated visually.

Table 2 Mineralogical zones for the mantle and their major mineral phases based on the quenched static high pressure and temperature experimental results. See text for reference for each phase transition

	Depth (km)	Major	Mineral	Phases
Upper mantle (zone B)	33		Pyroxene	
	Olivine zone	Olivine	+ Al ₂ O ₃	Garnet
Transition zone (zone C)	350	β-phase	Garnet	
			— Al ₂ O ₃	
	Spinel zone	Spinel	Spinel + Stishovite	Ferropericlasite + unknown (I)
	650		Ilmenite	
Lower mantle (zone D)	Oxide zone	Ferropericlasite + stishovite	Ferropericlasite + stishovite	Ferropericlasite + unknown (II)
	1,000			
	Perovskite zone	Perovskite + ferropericlasite	Perovskite	Perovskite
	2,800			

Phase transformations in this system, with increasing pressure, are inferred to be pyroxene → spinel + stishovite → ilmenite → ferropericlasite + stishovite → perovskite.

For the olivine system two samples of single phases of (Mg_{0.8}Fe_{0.2})₂SiO₄ and (Mg_{0.7}Fe_{0.3})₂SiO₄, which are identical to those used by Ringwood and Major², were studied. The quality of the X-ray diffraction data for the olivine sample of (Mg_{0.7}Fe_{0.3})₂SiO₄ are extremely good. A total of 52 reflections, which correspond to five crystal phases, has been observed. The lattice parameters are $a_0 = 4.774 \pm 0.008$, $b_0 = 10.354 \pm 0.016$, and $c_0 = 6.041 \pm 0.010$ Å for the starting sample of olivine; $a_0 = 8.143 \pm 0.007$ Å for the spinel phase; $a_0 = 4.278 \pm 0.003$ Å for ferropericlasite; and $a_0 = 4.805 \pm 0.004$, $b_0 = 4.949 \pm 0.004$, and $c_0 = 6.922 \pm 0.006$ Å for the perovskite phase of (Mg_{0.7}Fe_{0.3})SiO₃. These yield a molar volume of the perovskite phase of 24.78 ± 0.06 cm³ mol⁻¹ which is 4% smaller than that of its isochemical mixture of oxides.

The most abundant phases found in Fe₂SiO₄ (ref. 9) and Mg₂SiO₄ (ref. 10) are the rocksalt phase and stishovite. I found that ferropericlasite (rocksalt) and perovskite are the most abundant phases and only a trace amount of stishovite can be identified on the basis of the relative intensities of the various phases. I therefore conclude that nearly all the stishovite has reacted with ferropericlasite to form perovskite in the present experiment. On the basis of all the available evidence, the following phase transformations are inferred to have occurred in the olivine system: olivine → β phase → spinel → ferropericlasite + stishovite → ferropericlasite + perovskite with increasing pressure.

Similar results were obtained in the olivine sample of (Mg_{0.8}Fe_{0.2})₂SiO₄. Since this sample contained less iron than the previous one, it did not absorb the laser beam very well and was not well heated. Only about 30% of the sample has been transformed to the perovskite phase to judge from the relative intensities of the X-ray diffraction pattern. In a later run, however, the sample was intimately mixed with a few per cent of powdered graphite, which served to absorb the laser irradiation and to heat the sample under compression. The results show that sample to be about 80% transformed. The lattice parameters for the perovskite phase of (Mg_{0.8}Fe_{0.2})SiO₃ are $a_0 = 4.796 \pm 0.004$, $b_0 = 4.943 \pm 0.004$, and $c_0 = 6.902 \pm 0.006$ Å.

I would suggest that the mantle be subdivided on the basis of its major mineral phases or chemicals with depth, rather

than on the basis of velocity distributions (Table 2). Of course, the boundaries between the successive phases shown in Table 2 should in each case comprise a mixture of the phase above and that below the boundary for the ferromagnesian solid solution system. In view of all the experimental evidence, I conclude that the lower mantle is composed mainly of the perovskite phase, which may represent the last stage of the mineralogical evolution in the Earth's mantle.

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Ignition by shock wave focusing and staging of thermonuclear microexplosions

FROM the beginning of laser fusions research¹ the main problem has been the very large size of the ignition apparatus. For this reason relativistic electron beams² and, more recently, ion beams³ have been proposed as alternatives. Relativistic electron beams have the following principal problems, however, when compared with laser beams: first the pulse length is rather long, and second the beam focusing is not as good. Ion beams are not as easily focused as either laser or electron beams. Furthermore, in both laser- and electron-beam induced implosion most of the energy goes into the ablation products, rather than the thermonuclear target. I propose here an alternative, non-ablative energy deposition method, applicable to laser,

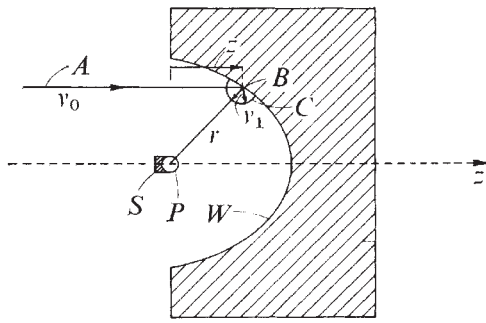


Fig. 1 A beam ray A hits a concave wall W , at point B . The wall material is ablated forming a small spherical shock wave C . The superposition of all the shock waves from all beam rays results in a curved shock wave, by Huygens' Principle. The beam consists of all the rays striking the curved wall and its total cross section is equal to the wall diameter.

electron or ion beams, and which has the following distinct advantages: (1) The energy is delivered to the target by a shock wave. (2) The beam energy can be focused precisely on the target. (3) The energy pulse length can be shortened by about an order of magnitude. (4) The method permits staging, whereby a small microexplosion can set off a larger one.

The principle of the idea is explained in Fig. 1. If the wall is properly shaped the resultant shock wave will converge on to the thermonuclear pellet (P). The pellet is protected by the shield (S) against the incoming beam, to prevent it from being preheated, which would hinder its compression to high densities. The shield simultaneously serves as an anvil for the pellet.

The wall shape is approximately determined by Fermat's Principle, and in a r - z coordinate system, with $r = z = 0$ at the pellet centre the wall shape would be given by

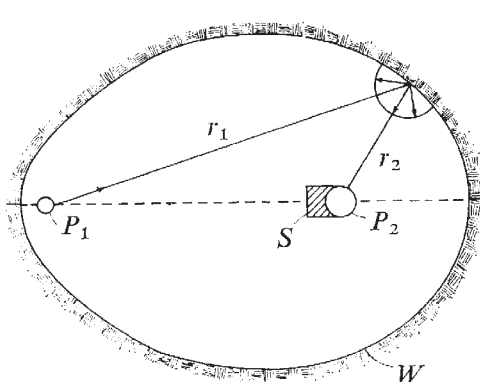
$$t = (z/v_0) + (r/v_1) = \text{const.} \quad (1)$$

where v_0 is the velocity of the incoming beam particles and v_1 the shock velocity. For relativistic electron beams $v_1 \ll v_0$, and hence the wall shape should be hemispherical. For ion beams, one may have $v_0 \approx v_1$, and the wall should be paraboloid.

By a proper layered variation of the density ρ and the atomic weight A in the wall material a shock wave is obtained which becomes radially compressed as the shock approaches the centre. If a is the initial distance from the centre of convergence of a particle immediately after the shock has been formed and if all the fluid particles arrive simultaneously at time $t = t_0$ at the centre of convergence (which is the condition for the radial shock wave compression), then the initial velocity of the particle in the shock wave must be

$$v(a) = a/t_0 \quad (2)$$

Fig. 2 A two-stage pellet. P_1 is the first stage, and P_2 is the second. S is a radiation shield to prevent P_2 from being preheated by the X rays emitted by the microexplosion of P_1 .



The particle trajectory is then given by

$$r = a(1 - (t/t_0)) \quad (3)$$

and the fluid density in the shock wave changes according to

$$\begin{aligned} \rho &= \rho_0(a/r)^3 \\ &= \rho_0(a)(1 - (t/t_0))^{-3} \end{aligned} \quad (4)$$

The energy flux density at fixed r , $r = r_1$, is then given by

$$\begin{aligned} \epsilon_1 &= (1/2)\rho v^3 \\ &= (1/2)\rho_0(a)(r_1/t_0)^3(1 - (t/t_0))^{-6} \end{aligned} \quad (5)$$

To achieve adiabatic high density compression the required pulse shape is

$$\epsilon \propto (1 - (t/t_0))^{-15/8} \quad (6)$$

This can be obtained by making

$$\begin{aligned} \rho_0(a) &\propto (1 - (t/t_0))^{33/8} \\ &= (r_1/a)^{33/8} \propto a^{-33/8} \approx a^{-4} \end{aligned} \quad (7)$$

If the temperature T in the shock wave is uniform, then the initial fluid velocity is

$$v^2(a) \propto T/A \quad (8)$$

where A is the atomic weight of the ablated material. But since $v(a) \propto a$ it follows that

$$A \propto a^{-2} \quad (9)$$

Assume an initial shock thickness of $\Delta a = 2$ cm, and an initial distance of the shock front from the centre $r_1 = 1$ cm; this would then require a variation in ρ of $\Delta\rho/\rho = (\Delta a/r_1)^4 = 16$, and a variation in the atomic weight by $\Delta A/A = (\Delta a/r_1)^2 = 4$. These variations in density and atomic weight can easily be met by putting several layers of different materials on the surface of the curved wall.

As the shock wave approaches the centre the energy flux profile remains self-similar and the energy flux density increases as $\epsilon \propto r^{-3}$, with geometric convergence contributing a factor r^{-2} , and radial wave bunching the additional r^{-1} . If the shock is focused from $r_1 = 1$ cm down to $r_0 = 0.1$ cm, the energy flux density increases by a factor 10^3 , and the pulse length is reduced by a factor 10. If an electron beam is used with a typical pulse length of 5×10^{-8} s, the reduced pulse length at the target would be only 5×10^{-9} s.

If the blast wave from one microexplosion is now focused by another solid wall on to a second thermonuclear target, a larger microexplosion can be ignited. In turn, the blast wave of the second microexplosion by reflection from a third wall can ignite a third, even larger microexplosion. The energy gain E_1/E_0 for one microexplosion is approximately given by (E_0 , E_1 in J)

$$E_1/E_0 \approx E_0^{1/3} \quad (10)$$

where E_0 is the input energy. For a TD thermonuclear reaction, only the energy going into charged fusion products can be utilised (which is about 20%). Assuming furthermore a coupling efficiency for this process of $\sim 50\%$ gives

$$E_1/E_0 \approx cE_0^{1/3}, \quad c \approx 0.1 \quad (11)$$

The energy gain in an n -stage microexplosion (allowing a factor 5 for the energy released by neutrons) is

$$E_n = 5 \sum_{n=0}^n c \sum_{m=0}^{n-1} (4/3)^m (4/3)^n E_0 \quad (12)$$

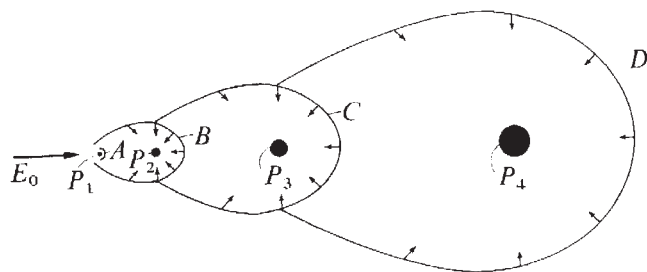


Fig. 3 A four-stage pellet. The incoming beam carries energy E_0 , and ignition P_1 . The wall A , cannot withstand the explosion of P_1 , which then is reflected by the wall B , to ignite P_2 , and so on.

If for example $E_0 = 10^4$ J, then a four-stage pellet would have $E_4 = 8 \times 10^6$ J. For $E_0 = 10^5$ J a two-stage pellet would already give $E_2 = 2 \times 10^7$ J. These values are at the threshold of reactor operation and show the great importance of staging in reducing the initial energy input.

Figure 2 shows a two-stage pellet, with both pellets located inside an egg-shaped cavity. The equation of the cavity wall is obtained from Fermat's Principle, and thus for the wall surface in bipolar coordinates r_1, r_2 centred at P_1 and P_2

$$(r_1/v_1) + (r_2/v_2) = \text{const.} \quad (13)$$

where v_1 is the velocity of the blast wave from pellet P_1 , and v_2 the velocity of the reflected shock. Figure 2 is drawn for the example $v_2 = (1/2)v_1$. To ignite P_1 any of the proposed methods can be used, including that proposed here. For this, however, the cavity must have openings near P_1 , to allow the passage of the original beams to ignite P_1 . A 4-stage pellet is shown in Fig. 3. The walls A, B and C are strong enough to withstand the initial blast wave but weak enough to collapse at the subsequent strong ones.

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Mechanism of corrosion protection in reinforced concrete marine structures

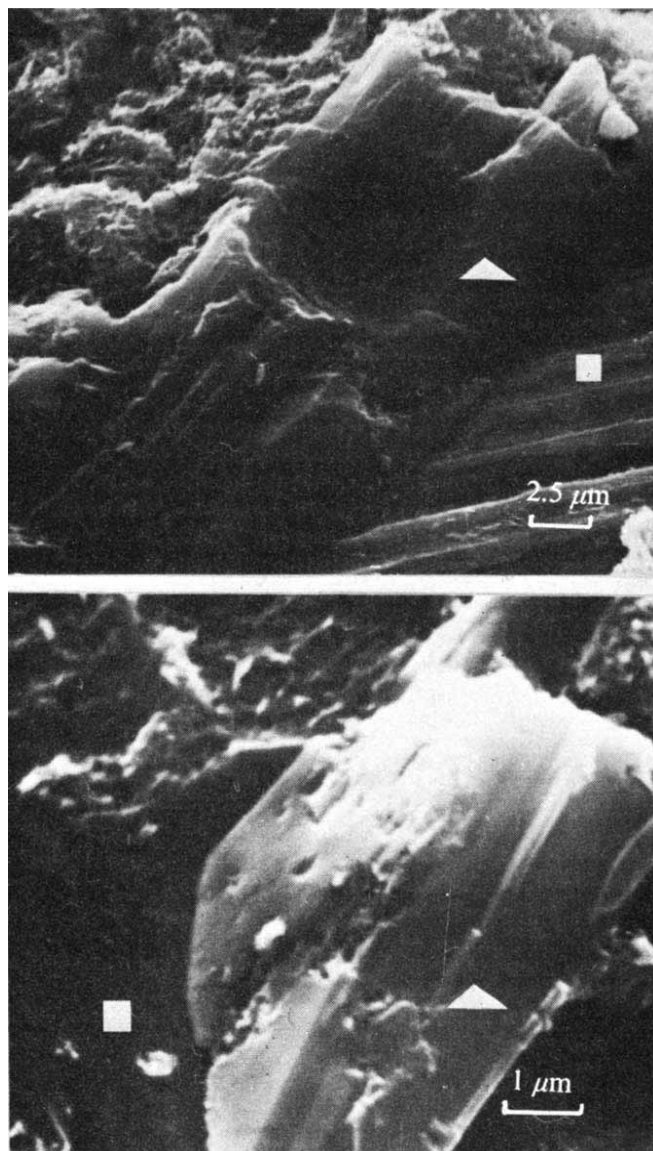
RECENT developments in concrete offshore structures have drawn attention to a disturbing ignorance of the mechanism by which steel corrosion is prevented when it is embedded in dense concrete¹. The traditional view has been that the metal remains passivated owing to the high pH (~ 12.5) of the pore solution in concrete. It is, however, well known that relatively small concentrations of Cl^- destroy the corrosion inhibitive properties of non-buffered alkalis and ~ 700 p.p.m. of Cl^- will depassivate steel in limewater at pH 12.5 (ref. 2). Since seawater contains concentrations of Cl^- far in excess of this figure, and since penetration by these ions to the reinforcing bars in concrete takes place within a small fraction of the service life of a marine concrete structure³, corrosion might be expected to be a frequent problem. In practice, however, there are many examples of reinforced concrete structures which have remained durable in seawater for 30 yr and more. Such corrosion problems as have been reported are, in the majority of cases, associated only with areas immediately above the level of high tide,

where the concrete is not maintained in a saturated condition.

These observations have led to an alternative suggestion that the main reason why saturated concrete suppresses the corrosion of steel is not its ability to promote anodic passivation but its low permeability to dissolved oxygen. Thus, although the ingress of Cl^- from seawater destroys the passive film on steel, a high degree of polarisation of the cathodic reaction prevents an appreciable corrosion current from being sustained⁴. Although this view surmounts the problem of Cl^- tolerance, it is by no means free from objections. It fails to provide an adequate explanation for the small number of cases in which corrosion of reinforcement has arisen in fully submerged marine concrete³. Furthermore, it has been observed that steel which is free from corrosion in concrete saturated with seawater is characterised by a high electrode potential, more positive than the passivation potential, and that the surface of the metal remains bright and apparently passivated⁵.

I suggest that these phenomena may be more satisfactorily explained if account is taken of the nature of the interfacial zone separating concrete and embedded steel reinforcement. Evidence has been obtained that this region of the material is composed largely of segregated

Fig. 1 Scanning electron micrographs of fracture surfaces showing interfacial lime layer (Δ) in contact with steel ($\square$).



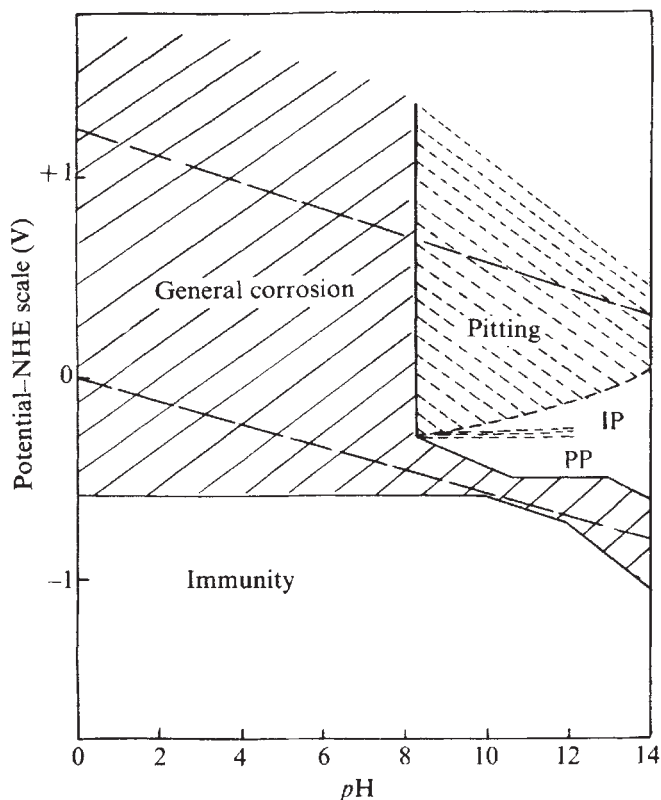


Fig. 2 Potential/pH diagram for Fe in 1 M Cl^- solutions. PP, Perfect passivity; IP, imperfect passivity

lime^{6,7}. Recent studies of the interface with a scanning electron microscope have confirmed this and shown the metal to be in intimate contact with a lime-rich layer over most of its surface (Fig. 1.) This layer will modify the electrode characteristics of the metal. The effect on the cathodic reduction of oxygen is apparent, since a considerable fraction of the metal surface is screened from the direct access of oxygen. The influence on the anodic behaviour of the exposed regions of the steel is also likely to be significant since the surrounding lime provides a reservoir of OH^- in direct proximity to the metal. Thus, the tendency for hydrolysis of anodic reaction products to depress the local pH will promote dissolution of the adjacent lime, thereby exerting a form of local buffering around the anodic sites.

It is established that anodic passivation of iron is greatly facilitated in the presence of alkaline buffers. For example, borate solutions at pH 9 are efficient corrosion inhibitors because their buffering action stabilises the anodic formation of $\text{Fe}(\text{OH})_2$ and $\gamma\text{-Fe}_2\text{O}_3$ which reinforces the passivating oxide film on iron at regions of localised weakness⁸. Solutions in which OH^- is the sole anion do not exhibit this behaviour at pH values lower than about 12 because of their lack of buffering capacity. In the presence of Cl^- , the effect of alkaline buffering may be understood in terms of the modified Pourbaix diagram for iron (Fig. 2)⁹. This shows that the domain of passivity covers a band of potentials, the width of which diminishes rapidly with decreasing pH. Thus, in concrete saturated with seawater, the buffering effect of the lime layer may preserve the conditions at a local anode within the confines of this domain and, in view of polarisation of oxygen reduction, it is likely that the potential will be maintained in the range of 'perfect passivity' where pits in the oxide film are unstable. Corrosion is normally observed only in conditions where the material is subjected to alternate wetting and drying when evaporation can produce very high local salt concentrations and intimate contact between the lime layer and the metal may break down.

If the postulated role of the interfacial zone is accepted, several other practical observations become readily explainable. Measures which promote homogeneity of the interface, such as the application of a slurry of neat cement paste to the surface of reinforcing bars before surrounding them with concrete, are effective in reducing the risk of corrosion¹⁰. Conversely, it has been found that voids at the interface are almost invariably the positions at which corrosion of steel reinforcement and prestressing tendons takes place in the presence of high Cl^- concentrations¹¹. Furthermore, since the lime component of hydrated Portland cement is vulnerable to carbonation, leaching and attack by ions such as SO_4^{2-} and Mg^{2+} , the integrity of the interfacial zone depends on its being shielded from these forms of degradation by surrounding the reinforcement with an adequately deep covering of good quality concrete. This is the time-honoured method of providing corrosion protection to reinforcing steel in concrete. It is noteworthy that reported instances of reinforcement corrosion in concrete, fully submerged for extended periods in seawater, have been accompanied by observations of substantial chemical attack of the hydrated cement paste¹².

Further experiments are in hand to establish the effect of the interfacial layer on the electrode characteristics of steel in concrete, and an account of factors affecting the micro-structure of the interfacial zone will be published elsewhere (M. Al Khalaf and C. L. Page, in preparation). It is important that the fundamental basis for the corrosion behaviour of this system should be more fully understood so that rational assessments may be made of the potential risks of damage to concrete offshore structures. In particular, it is conceivable that the effects of cumulative fatigue loading due to wave action on the highly stressed elements, which are a novel feature of current designs, may stimulate gradual chemical degradation and leaching of the interfacial lime layer. The possible consequences of this on the fatigue life of the reinforcing bars are not readily predictable from accelerated corrosion fatigue tests of the types presently being conducted⁵.

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Conodonts as indicators of palaeogeographic regimes

CONODONTS are disjunct elements of, probably, an internal food-gathering and/or macerating device of the conodontophorid animal which flourished in the Palaeozoic and early Mesozoic seas. They are excellent tools for biozonations and long distance correlations of marine sedimentary rocks of that age¹.

Here, we draw attention to the potential of conodonts as tools for palaeogeographic analyses of marine depositional environments. Ordovician conodonts can be used in studies involving the extent and relative depths of sedimentary basins; for unravelling patterns of transgressions and regressions; as aids in the recognition of depositional environments characterised by raised temperature and salinity; and in understanding major palaeogeographic and tectonic changes. These new aspects of conodont studies are based on the hypothesis that the majority of conodontophorids were nekto-benthic organisms² rather than pelagic³.

Two major ecological models for the conodontophorids have been proposed (Fig. 1a and b). The first³ (Fig. 1a) suggests that the conodontophorids were pelagic free-swimming organisms with certain taxa exhibiting depth stratification in the water column. Thus, nearshore deposits would yield only surface-dwelling forms, and progressively deeper water facies would yield surface-dwelling forms plus elements from deeper 'stacked' biofacies. The second model (Fig. 1b), which we favour, suggests that the conodontophorids were primarily nekto-benthic and laterally segregated into series of well defined communities; this model is applicable to all major types of Ordovician marine depositional environments², to epeiric sea deposits, and to marginal cratonic basin and continental margin deposits. Studies of Carboniferous⁵, Silurian, and Lower Devonian conodonts have indicated similar patterns of distribution and we believe our model to be generally applicable to the majority of conodontophorids existing from the Ordovician through to the Triassic. Cambrian forms we are still uncertain about.

Samples from epeiric-sea deposits frequently indicate extensive overlap between neighbouring conodontophorid communities (Fig. 2). We believe that occurs because of the exceedingly gradual depth changes of the epeiric sea. We also believe, however, that the inherent time-average nature of the conodont sample is a contributory factor. Whether a conodont collection will represent more than one community is of course directly dependent on rates of sedimentation, stratigraphic thickness of the sample, and, ultimately, the stability of the depositional environment. This is of particular importance in studies of conodonts

digested from starved sequences such as the Lower and Middle Ordovician of Sweden where net average sedimentation rates of less than 5 mm kyr⁻¹ have been estimated^{6,7}.

We believe the overlap of neighbouring conodontophorid communities can be used to identify minor transgressions and regressions in a sequence of strata simply by comparing the proportions of those living in shallow water with those in deeper water (see Fig. 2). We have compared the proportions of conodont elements from offshore living *Phragmodus* with those of nearshore living *Plectodina* from a series of samples from two sections of the Champlainian and early Cincinnati Lexington Limestone of Kentucky. The data are based on numbers of conodont elements tabulated by Bergström and Sweet⁸.

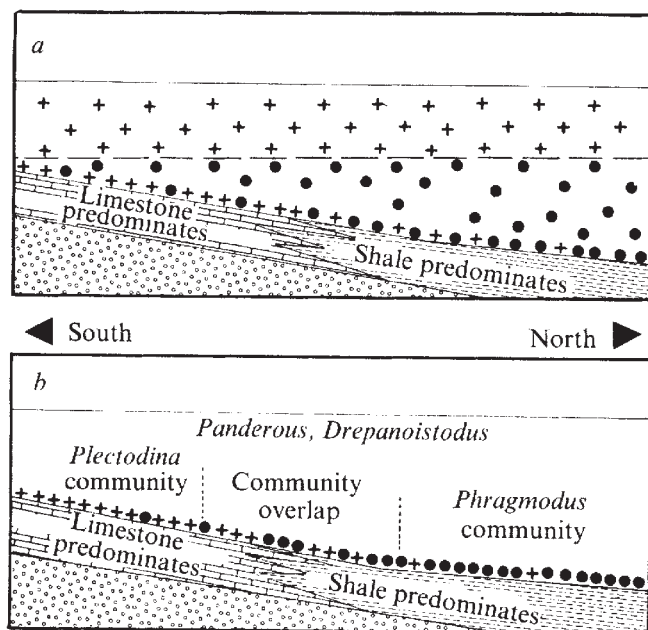
The *Phragmodus* apparatus includes four different elements (Fig. 2aI) whereas the *Plectodina* apparatus includes six different elements (Fig. 2aII), so the numbers could be considered biased in favour of the *Plectodina* community. Though the exact numbers of elements in each of these two apparatuses are not known, only the proportional distributions and trends through time are of interest (Fig. 2b and c). A moving average minimises the element of chance (in this case samples with possible artificially biased conodont collections), and emphasises the trend.

If we are correct in our assumption about the nekto-benthic habit of *Phragmodus* and *Plectodina*, then the resulting curves of proportions of *Phragmodus* elements should directly reflect transgressions with increasing percentage and regressions with decreasing percentage. If such a relationship existed it should show up as a clearly displayed trend; (this, of course, with the proviso that sampling intervals are not too large and sedimentation rates are not too slow). Such trends are clearly visible in Fig. 2 (for example, the initial transgression in section 64S3 (Fig. 2c), which is based on six samples showing almost a linear relationship, or the final major transgression interspersed with a series of minor regressions in the Clays Ferry section (Fig. 2b)). If the conodontophorids were pelagic the plotted values would be unevenly distributed with a number of unrelated maxima and minima.

The Lexington Limestone is a bioclastic calcarenite with a varying amount of argillaceous admixture and shaly interbeds, and exhibits frequent facies changes and recurrence of facies types⁹. In other words, it is characterised by numerous fluctuations in water level¹⁰. Bergström and Sweet¹¹ noted that *Phragmodus* is more common in the shaly intervals, and Seddon and Sweet³ have suggested that the ratio between *Phragmodus* and *Plectodina* provides a crude index to relative water depth. We believe that ratio can be used with much more reliance and precision; that is, to determine possible changes in water level, bed by bed in a section.

A more stringent test of our model is provided by an examination of a deeper basin of considerable size to determine what the supposed surface-dwelling forms also accumulate with those deeper in the water column. Chamberlain and Clark¹² have studied conodonts and trace fossils from the Oquirrh Basin of central Utah where up to 8,000 m of sediments accumulated during the Pennsylvanian and early Permian on the eastern margin of the Cordilleran geosyncline. The sediments and the trace fossil sequence—*Cruziana* to *Zoophycos* to *Nereites*—indicate a progressive increase in water depth for the central part of the basin. Forty-five samples from shallow water bioclastic limestones of the *Cruziana* facies yielded 7,000 conodonts, dominated by *Adetognathus* (high population; low diversity). Forty samples from limestones in the deeper *Zoophycos* facies yielded only 1,000 specimens, dominated by *Idiognathus* with some *Adetognathus* species (few individuals; high diversity). From micritic carbonates and siltstones in the deep basin *Nereites* facies, 45 samples produced only a few fragmentary conodonts—excluding some introduced by

Fig. 1 Contrasting models of conodontophorid habit: a, pelagic; b, nekto-benthic. Arrow, sea level; +, *Plectodina*, *Icriodella* and *Panderodus*; ●, *Phragmodus*, *Amorphognathus* and *Belodina*. We prefer model b.



turbidity currents from shallower environments—(individuals rare to absent). These data demonstrate the usefulness of conodonts in identifying specific depth regimes in ancient basins. Moreover, the virtual absence of conodonts from the deeper regions of the basin, estimated to be about 500–700 m deep, and the marked lateral variation in faunal composition with little mixing indicate that the conodontophorids had a nektonic habit.

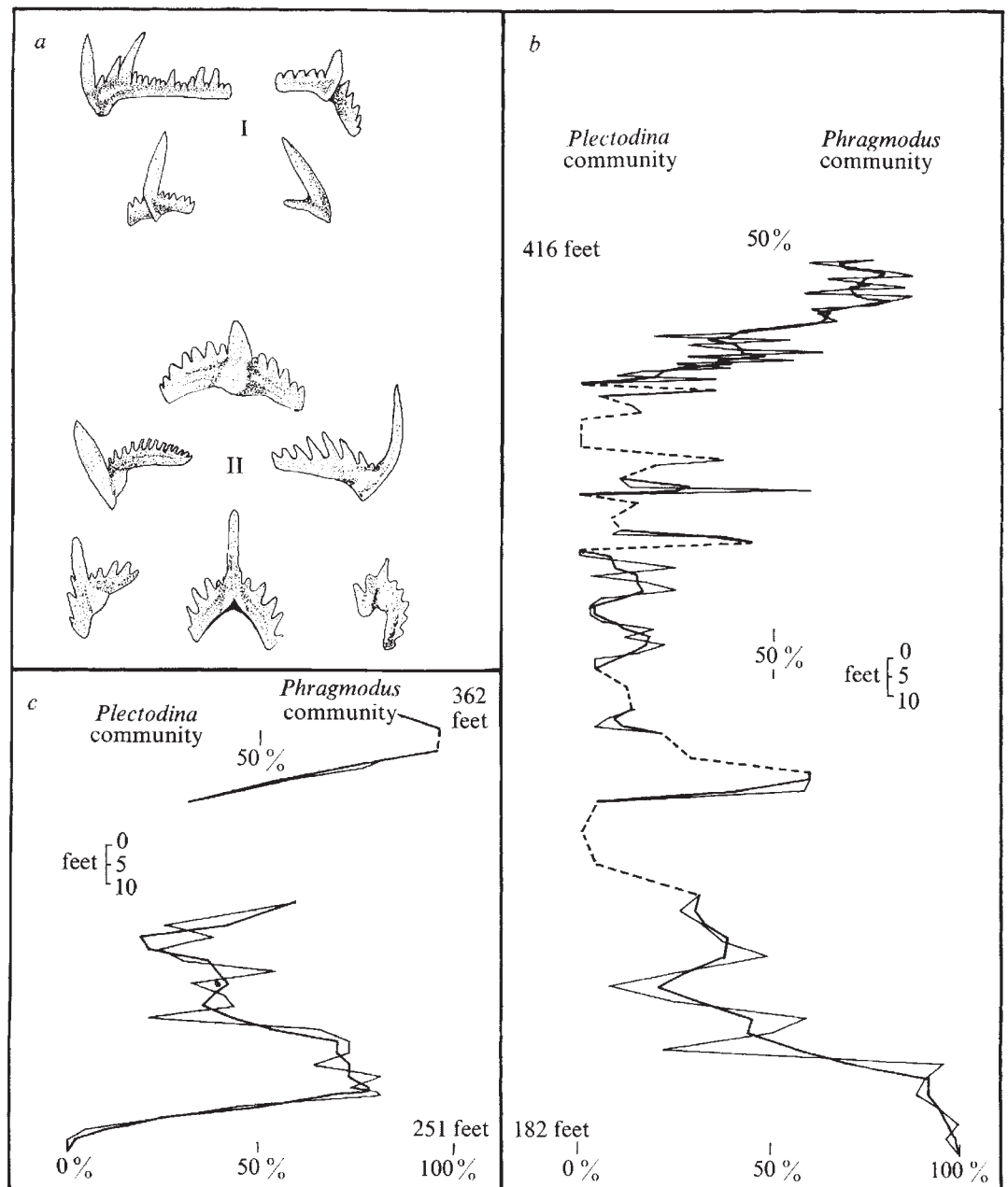
The early phases of closing of the proto-Atlantic Ocean are, on the Laurentian Plate, (at least in part), first noticeable in western Newfoundland¹³. The first indication of the destruction of the continental margin is provided by the upheaval of the carbonate shelf at the end of the early Ordovician, which resulted in karst topography developing on the carbonate bank (St George Formation) situated at the outer edge of the shelf¹⁴. This upheaval cannot be dated with accuracy using fossils extracted from the bank as they are relatively long ranging and many are as yet undescribed. It can, however, be dated with fossils from the deposits of the continental slope as the slope was elevated but never subaerially exposed, and also by fossils from the initial easterly derived clastic material from the klippen.

The middle Cambrian–early Ordovician Cow Head Breccia of western Newfoundland is considered¹⁵ to be a continental slope deposit with clasts derived from the edge of the shelf (St George Formation) and the uppermost part of the continental slope (lower Table Head Formation).

Ordovician conodonts extracted from limestones interbedded with the breccia and from the matrix are typical of the North Atlantic Province¹⁶. Lower Arenigian deposits are characterised by the genus *Prioniodus* whereas Upper Arenigian deposits are characterised by *Periodon*. These genera are typical of two neighbouring conodontophorid communities, with the former placed further offshore².

During middle Arenigian time the environment of deposition of the breccia seems to have become suddenly shallower; this is particularly well displayed in the conodonts which, with practically no overlap, change from typical *Prioniodus* to *Periodon* faunas¹⁷. This abrupt changeover occurs in the upper part of the *Prioniodus evae* zone, which correlates with the *Didymograptus nitidus* subzone of the British standard graptolite zonation¹⁸. Graptolites collected immediately to the south of Cow Head from flysch deposits derived from the incoming klippen also indicate the

Fig. 2. *a*: I, Conodont elements of the *Phragmodus* apparatus; II, conodont elements of the *Plectrodina* apparatus. *b*, Percentage of *Phragmodus* elements in the total of *Phragmodus* and *Plectrodina* elements in a section at Clays Ferry, Madison, County Kentucky (ref. 8, section 60P)—the section is 84 m thick and a total of 128 samples yielded 14,656 conodonts. Fine line, actual percentage; thick line, percentage based on a moving average calculated on every set of three samples starting from the base of the section; dashed line connects numbers where intervening samples contained no conodonts or a number of conodonts considered too small to be statistically significant (20 specimens were arbitrarily chosen as the cutoff limit.) *c*, As for *b* but Section 64S3 of ref. 8. Section about 41 m thick, and a total of 32 samples yielded 8,978 conodonts.



D. nitidus subzone. Thus, initial destruction of this ancient continental margin can be dated with considerable precision. Detailed stratigraphic implications of this will be published elsewhere.

Distinctive conodonts are also restricted to extremely shallow water environments. A prime example is provided by the neurodont (fibrous, hyaline) conodonts of the Middle Ordovician. They form a littoral community found in intertidal and lagoonal carbonate environments which probably experienced both high temperatures and salinities². Their virtual absence in deeper shelf facies also suggests a benthic habit³. Higher energy environments, developed as local shoal banks or around monadnock islands are characterised by more massive, robust elements. The niches occupied by these neurodonts were largely destroyed with the complete submergence of the North American craton during the late Ordovician, thus explaining their extinction.

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Flatworms afloat

ON March 1, 1975 we sighted from the air, on the surface of the sea near La Paz, Baja California (Mexico), some unusual reddish brown streaks, extending for several hundred metres obliquely to the shore. On the following day we relocated them by ship (RV Dolphin) and found them to consist of high densities of the dinoflagellate *Noctiluca* and of a polyclad turbellarian, provisionally identified as *Stylochoplana sargassicola* (Mertens). Although blooms of *Noctiluca* are not uncommon¹, marine pleustonic flatworms in large numbers are virtually unknown.

Between March 2 and 10, 1975 we made about 20 neuston tows across an area between 22°N and 25°N, and between the western coast of the Baja California peninsula and 110°W. We used a neuston net with fine nylon mesh cod end (202 µm pore size) supported by floats, and sampled swaths of the ocean surface 1 m wide (mean depth 10 cm, depending on wave size) and several hundred metres long. Samples from the brown patches in the La Paz harbour area proved to consist largely of *Noctiluca*, with an admixture of other dinoflagellates; it is probable that the latter were responsible for the brown colour, but that we collected relatively few of them because they were of a size to pass through the pores of the net. We were surprised that samples from all the other brown patches sampled on subsequent days consisted almost exclusively of brown flatworms, associated with more typical pleuston organisms² such

as *Porpita*, *Physalia*, *Glaucus*, *Ianthina*, *Pleurobrachia*, various smaller copepods, amphipods, decapod megalopa and fish larvae, and, in some tows, an abundance of terrestrial insects. The flatworms were especially numerous in tows taken between 24°20'N and 23°50'N, in some cases clogging the net. A rough calculation indicated that in one area, sampled on March 8, 1975, the surface density of these worms reached 1 m⁻². They decreased markedly in abundance south of this zone, and were absent altogether from samples taken south of 23°N.

The animals, of all sizes from 2 to 12 mm long, were beautifully camouflaged among the leaflets of the brown alga, *Sargassum* sp., with which they were often associated. Since the green or brown colorations of certain marine turbellarians, notably species of *Convoluta*, are attributable to the presence of symbiotic algae (respectively, *Platymonas* and *Licmophora* spp.^{3,4}), we suspected that the brown granules which constituted the speckles in the body wall of our worms might be symbiotic diatoms or dinoflagellates. They were, however, much too small, too dense and too irregular in shape, and we concluded that they were granules of animal pigment. Unlike dinoflagellates (or *Sargassum*), they did not turn green when denatured by boiling, drying or freezing, and did not yield a green solution when the animals were extracted with acetone, so we dismissed the possibility that they were, or contained, chloroplasts.

The animals displayed three quite distinct modes of movement. (1) They could glide (a few mm s⁻¹), presumably by ciliary movement, on solid substrates (such as seaweed, fish or ctenophores) or on a relatively undisturbed air-water interface. (2) They could swim slowly, in a horizontal position, by undulations of the lateral margins of the anterior two-thirds of the body. (3) Alternatively they could progress much more rapidly by turning on one side and swimming, like a tadpole or small fish, with serpentine undulations, one or two per body length. This mode of progress was presumably noted for *Planaria pellucida* by Mertens⁵, who described it as "geschlaengelt" and likened it to the swimming movement of polychaete worms.

They readily settled on most solid substrates (weed, net, bucket and so on) from which they could be dislodged only with difficulty. (They seemed to prefer not to settle on *Porpita*, being perhaps repelled by its stinging nematocysts.) Specimens were rarely observed to be attached by only the posterior end, while the rest of the body swayed freely in the water. They were not seen feeding.

Clusters (3-6 mm diameter) consisting of hundreds of white eggs (100 µm diameter), which may have been laid by these worms, were found attached to the surface of *Sargassum* thalli collected in the same tows. They contained embryos, consisting of a few cells, or early blastulae; their further development was not observed.

Although many marine littoral turbellarians have been described, there have been few reports of truly pelagic species. Hyman⁶ mentioned none in her review of marine turbellarians of the Pacific coast of North America. We have been able to trace in the literature several records of pelagic flatworms, but their taxonomy seems to be rather confused. Our specimens closely resemble the polyclad flatworm *Stylochoplana sargassicola* (Mertens)⁷ described from the Sargasso Sea by Mertens⁵, who found it associated with the seaweed, *Sargassum bacciferum*. It is cryptically coloured, being translucent and speckled with pale yellow or yellowish grey pigment granules. This worm is not confined to the Sargasso Sea, however; it has been found elsewhere in the Atlantic Ocean between 21-35°N and 36-38°W. This species may be synonymous with *Stylochus pelagicus*, described by Mosely⁸ from specimens collected along with *Halobates* and other pelagic animals off the coast of Sierra Leone. On both occasions when this worm was collected (August 1873, between 9°21'N 18°25'W and 5°48'N 14°20'W) the sea was highly phosphorescent, presumably, as in our own case, due to large numbers of dinoflagellates such as *Noctiluca*. Apparently *S. sargassicola* has never been reported from the Pacific Ocean before.

Von Graff⁷, who referred the nine described pelagic turbellarians to three species (and who also described two new species), recorded another flatworm, *Planaria pellucida* Martens, from the Atlantic as well as the Pacific Ocean. This species, originally described as *P. oceanica* by Charles Darwin⁸, was found about 150 miles from Natal, Brazil (5°S 33°W) in February, 1844. Mertens⁵ collected his specimens about 500 miles offshore at 7°48'N 23°56'W, where they occurred together with typical pleustonic organisms such as *Porpita*, *Glaucus* and *Physalia*. This species is also somewhat transparent, but the body is whitish yellow, without evident spots. Our specimens seem to be morphologically quite different from *P. pellucida*.

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Production of threitol and sorbitol by an adult insect: association with freezing tolerance

WE report here that in adult tenebrionid beetles, *Upis ceramboides*, an unusual combination of two polyhydric alcohols, sorbitol and threitol, is associated with the ability to tolerate prolonged freezing to at least -50°C . The occurrence of threitol is of particular interest, since this compound has not previously been found in nature¹.

Following the discovery that immature insects may produce glycerol during diapause², polyhydric alcohols, usually glycerol, have been implicated as cryoprotective agents in a variety of overwintering insects^{3,4}. Because of its relatively small molecular size, glycerol is able to penetrate most cell membranes, and its ability to prevent freezing damage is generally believed to lie in its colligative properties and its ability to act as a solvent, keeping potentially harmful salts in solution as they are concentrated during ice formation⁵.

Although non-penetrating compounds, including sorbitol and mannitol, are reported to have cryoprotective action on isolated cells⁶ there are no reports of such a function in whole insects. Sorbitol has been found in the eggs of two insects^{6,7} and in the larva of the dipteran *Eurosta solidagenis*⁸. The latter insect is tolerant to freezing but the function of sorbitol is obscured because glycerol is also present. The only other polyol reported to occur in insects is mannitol, which occurs with glycerol in the eggs of the overwintering aphid *Hyalopterus*⁹.

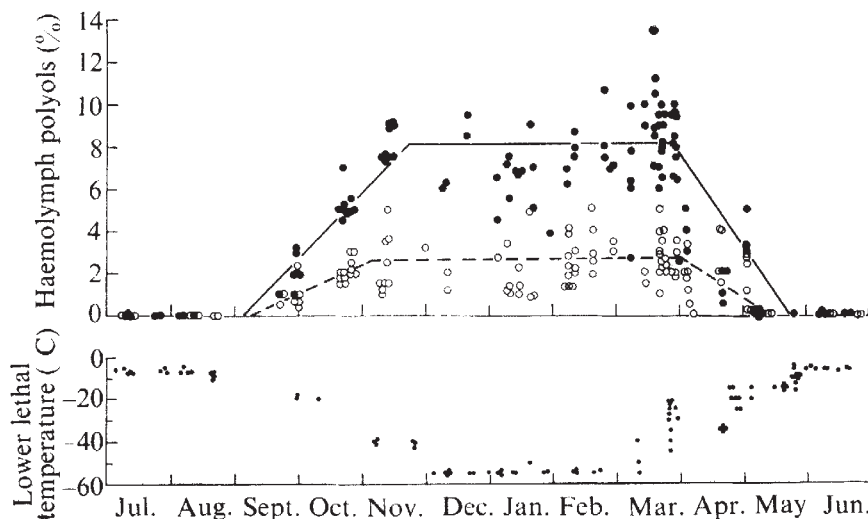
Freezing tolerance in an adult insect was first documented in a carabid beetle, *Pterostichus brevicornis*¹⁰, and is associated with the accumulation of large quantities of glycerol¹¹. A more extensive survey of overwintering northern insects has revealed a number of freeze-tolerant species (L. K. M., unpublished), but *Upis ceramboides* is of particular interest because its large size (~ 150 mg) makes analysis of a variety of haemolymph constituents in each individual possible. It overwinters in a very exposed habitat above the snowline, and, as we show here, synthesises large quantities of polyols other than glycerol.

Adult *Upis* may be found throughout the year in interior Alaska and Canada beneath loose bark of aspen, birch, and spruce. During winter, air temperatures in these regions may fall to -50°C and they often remain below freezing point for many months. Beetles collected during winter and brought into the warm laboratory recover their normal locomotion and frequently begin to feed within several hours. The relative scarcity of *Upis* has made it necessary to accumulate data over a 4-yr period to obtain sufficient seasonal information. In addition to analysis of haemolymph polyols, information was obtained on lower lethal temperatures, supercooling points and other physicochemical characteristics not included here. Because of the temperature lability of insect polyol levels, care was taken to perform all tests less than 1–2 h after specimens were brought into the laboratory. If specimens had to be stored, they were kept outdoors in conditions approximating natural ones.

Supercooling points have been determined using a thermoelectric technique on a large number of specimens and average -6.3°C , which is quite high for a cold hardy insect and, surprisingly, shows no significant seasonal variation in spite of changing polyol levels. Initial tests for polyhydric alcohols using paper chromatography with several solvent systems showed that no glycerol was present but that sizeable amounts of either sorbitol or mannitol, and what was believed to be erythritol, were present during the winter months. Polyol levels drop to undetectable amounts in the summer and build up during the autumn (Fig. 1).

To identify whether it was sorbitol or mannitol that was

Fig. 1 Haemolymph concentrations of threitol (---, ○) and sorbitol (—, ●) during the year and their relationship with lower lethal temperatures in *Upis ceramboides*. Data points represent individual values obtained over a 4-yr period from 1970–71 to 1973–74. Polyol levels were determined using paper chromatography with a solvent system of butanol-acetic acid-water (12:3:5 v/v), and periodate followed by borate-starch for detection. Positive identification was by GLC using a 150 cm $\times$ 3 mm glass column with 10% SP-2401 on Supelcoport 100/120, 220 $^{\circ}\text{C}$ column temperature. Lower lethal temperatures represent 100% mortality following a 1-h exposure to the final temperature. Cooling rates were $0.3^{\circ}\text{C min}^{-1}$.



being produced by *Upis*, gas-liquid chromatography (GLC) of alditol acetates of the polyhydric alcohols was used^{12,13}. It was found that sorbitol is produced, not mannitol, but the compound previously identified as erythritol was found to have a slightly greater retention time than the erythritol standard. On the basis of previous work¹⁴ on the separation of acetylated derivatives of tetritols we concluded that the unknown polyol must be threitol. A sample of threitol was obtained as a standard and compared with *Upis* fat-body extracts and individual and mixed standards of glycerol, sorbitol, and (meso) erythritol using GLC^{12,13}. Distinctly separate, well defined peaks were obtained for each polyol in the standards and extracts. The tetraacetate for our D-threitol standard consistently showed identical retention times with the unknown polyol in *Upis* fat-body extracts, and the threitol standard and the polyol in the fat-body extracts invariably had retention times that were 15% longer than the erythritol standard. Although the threitol standard we obtained was D-threitol, the GLC technique cannot distinguish D and L forms on the basis of retention times, so we can only be sure that the polyol in *Upis* is an enantiomer of threitol, and is not erythritol.

The wood-rotting fungus *Armillaria mellea* has been reported to produce threitol on an artificial glucose medium¹⁵, so it was possible that the beetles had been feeding on rotten wood containing *Armillaria*. To test this possibility, a group of *Upis* were deacclimatised at room temperature until their haemolymph polyols dropped to zero. The beetles were then cooled in stepwise fashion over a period of weeks and tested at intervals for polyols (Fig. 2). It is evident that the cold reacclimatised beetles were able to produce sorbitol and threitol in response to the artificial cold stress.

On the basis of the correlation between haemolymph polyol levels and freezing tolerance (Fig. 1) it seems that either sorbitol or threitol, or both, have a cryoprotective function in *Upis ceramoides*. This is the best evidence to date that polyols of higher molecular weight than glycerol can serve as natural cryoprotectants. This is the more unusual because it applies to a large, adult insect with its inherently greater tissue complexity and susceptibility to temperature gradients in the body during ice formation. Since threitol contains only one more carbon atom than glycerol, it may be able to penetrate the cells of *Upis*. Indirect evidence for this is provided by the very low cooling rates, 0.3 °C min⁻¹ or lower, which are required to prevent freezing damage at temperatures below -30 °C (L. K. Miller, unpublished), and that penetrating cryoprotective agents are typically associated with protection against slow freezing injury¹⁶.

Because of its higher molecular weight, the function of

sorbitol in freezing tolerance is more difficult to comprehend, although the present study provides stronger circumstantial evidence for its importance. If sorbitol does act as a non-penetrating cryoprotectant, its very high concentration in *Upis* haemolymph is somewhat of a mystery since non-penetrating agents are generally effective at very low concentrations⁴.

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Endogenous rhythm of nerve activity in the housefly eye

WHEN a glass electrode filled with KCl is placed on the eye of a housefly bursts of nervous activity occurring even in darkness may be observed using an oscilloscope. We present evidence that the diel changes in burst frequency represent a true circadian rhythm. The source of these signals obtained from the housefly eye is as yet unknown. Dethier¹ reported two types of electrical activity as recorded externally from the insect eye, the electroretinogram (ERG) and spontaneous nerve firing. The latter activity was related to light intensity and movement in the visual field.

An endogenous circadian rhythm in the rate of nerve firing in darkness from the housefly eye is not inconsistent with current information on insect circadian rhythms as reviewed Brady². Other physiological activities of insects are reported to have at least diel fluctuations. The only previous reports of daily fluctuations in the electrical response of arthropod visual units were both concerned with responses to light. Jahn and Crescitelli³ suggested a diurnal change in the ERG of a carab beetle, and Aréchiga and Wiersma⁴ found a circadian rhythm in the response of the crayfish optic nerve to light.

In our experiments, flies with an active KCl electrode in contact with the corneal surface of the eye were shielded in a light-proof metal box. Neural activity was monitored at hourly intervals on an oscilloscope and the discrete bursts of spikes (Fig. 1) were counted. Recordings from individual flies reared in darkness and handled only in red light could be made over several periods of 24 h or more. The rate of spontaneous nervous activity in the single housefly eye over 24 h is shown in Fig. 2a. The rhythm is monophasic with a period of approximately 24 h. The rhythms of individual flies monitored over the same 24 h period showed peaks at random times. The rate of nerve activity during the peak periods was 10-20 times greater than base levels. The period of increased activity lasted 8-12 h.

Fig. 2 Changes in haemolymph concentrations of threitol (---), and sorbitol (—) at different acclimatisation temperatures. Note the disappearance of polyols after exposing winter-adapted beetles to 23 °C for only 3 d, followed by the more gradual buildup of alcohols during subsequent cold exposure.

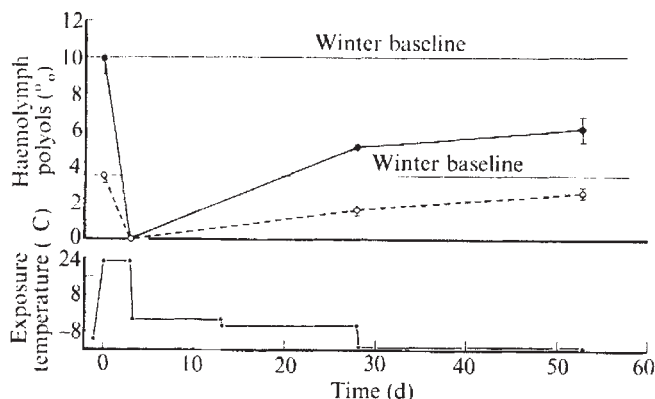


Fig. 1 Bursts of impulses recorded externally from the housefly eye in darkness (trace speed: 200 ms per division).

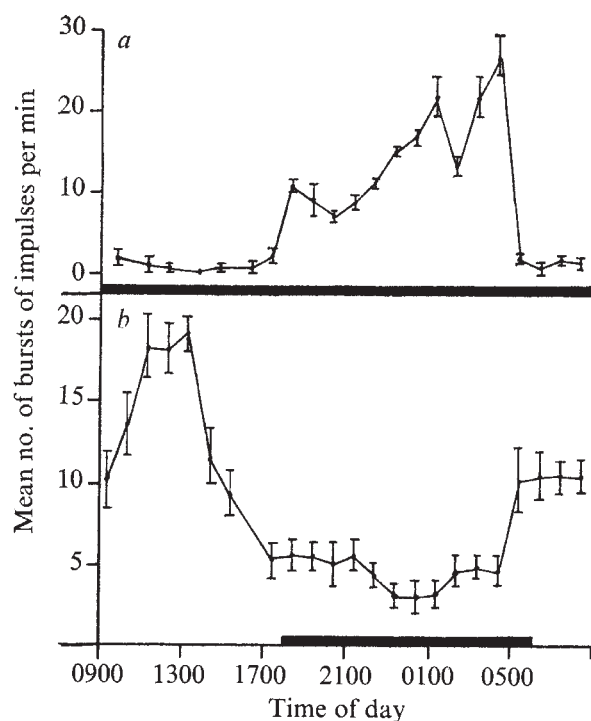
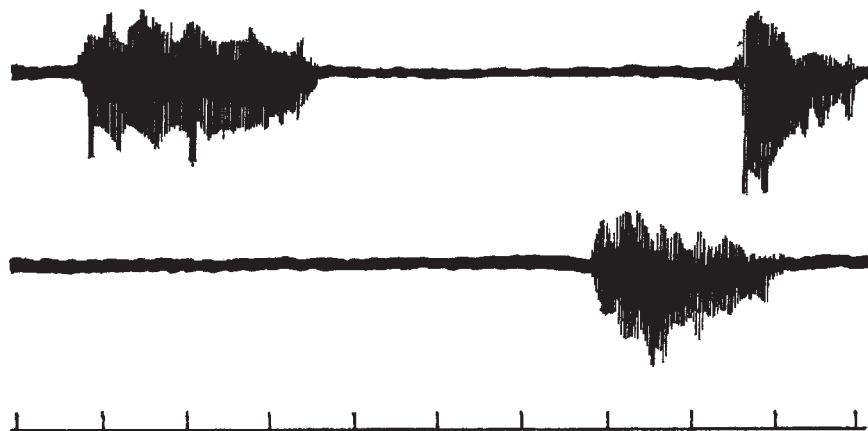
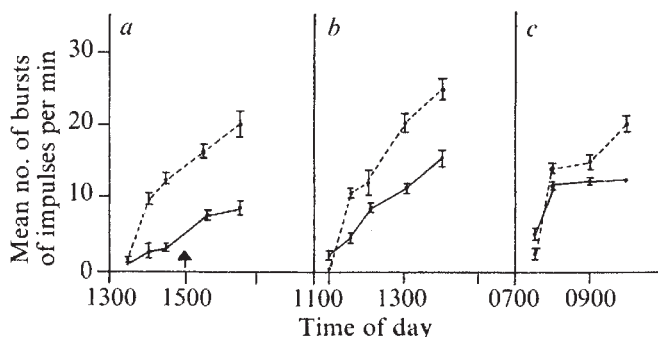


Fig. 2 Variation over 24 h in mean hourly rate of nerve activity in darkness from *a*, single housefly eye; *b*, from groups of houseflies reared in light-dark 12:12, photophase 0600–1800. Bars represent s.e.

Fig. 3 Mean rate of eye nerve activity in darkness, from two houseflies, over the time of the start of the activity peak on three successive days (bars indicate s.e.). Flies reared dark-dark. Nerve activity rhythms were synchronised by an entraining photophase, 1500–2200, the day before day 1 (*a*). On day 1 flies anticipated "dawn" (indicated by arrow) but thereafter the flies displayed a free running period. *b*, Day 2; *c*, day 3.



We also have evidence that the rhythm can be entrained by a photoperiod (Fig. 3) and has a free-running period of 20–22 h. Experiments involving mean hourly responses of groups of flies reared in light-dark 12:12 and 16:8 artificial light photoperiods indicated that the flies were able to anticipate "lights on" (Fig. 2*b*). Results showed the beginning of the peaks of nervous activity were synchronised to start about half an hour before dawn.

Our experiments are continuing and the details of our findings will be reported elsewhere.

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Recognition of self from non-self in crustaceans

THE immune response of vertebrate species seems initially to involve recognition events between immunogenic molecules and specific lymphocyte receptors. These recognition events subsequently result in amplification steps including, in many cases, increases in cells capable of producing humoral antibodies or participating in cell mediated reactions¹. Since invertebrates apparently cannot make humoral antibodies^{2,3} but can reject allografts^{4,5} it seems appropriate to inquire if such animals have receptors capable of discriminating self from non-self. The similarities between antigen receptors and humoral antibodies in vertebrates^{6,7} serve as additional reasons for investigating recognition mechanisms in invertebrates. Although sufficient data exist to indicate that the lectin-like agglutinins present in haemolymph can function as opsonins for foreign erythrocytes in certain invertebrates⁸, considerations of foreignness of protein molecules are not as definitive. Certain crustaceans, such as crayfish⁹ and lobsters¹⁰, can readily clear certain mammalian serum albumins from the circulation but little is known of the specificity of this elimination. We have investigated whether crustaceans possess recognition molecules (referred to as receptors) enabling them to recognise various proteins as foreign and to eliminate these proteins from the circulation. The basic premise was that if such receptors are present, regardless of their location (cell-bound or free), they should be of finite number (and thus saturable) and have some discernable degree of specificity.

Table 1 Distribution of radioactivity in crayfish tissues 2 h after injections of ^{125}I -BSA

Tissue	Tissue weight	Animal 1		Tissue weight	Animal 2	
		Counts per 0.1 min per g tissue	Counts per 0.1 min per total tissue		Counts per 0.1 min per g tissue	Counts per 0.1 min per total tissue
Gills	2.6	51,000	132,600	2.2	50,000	110,000
Heart	0.2	2,500	500	0.5	2,000	1,000
Hepato-pancreas	1.6	2,800	4,480	1.6	500	800
Digestive tract	1.1	1,200	1,320	1.1	1,600	1,760
Green gland	0.12	30,000	3,600	0.3	59,000	17,000
Muscle	5.3	1,800	9,540	4.0	3,000	12,000
Exoskeleton	18.0	100	1,800	19.0	1,000	19,000
			Total 153,800			161,560

*Each animal received 200,000 counts per 0.1 min at $t = 0$ h.

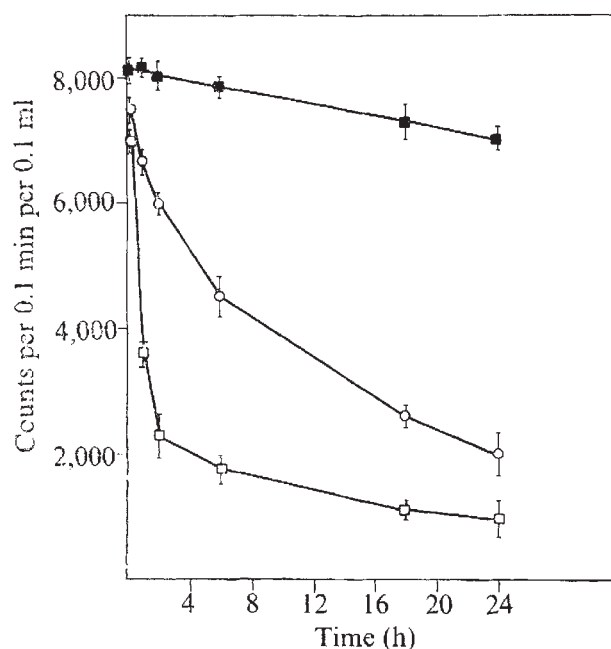


Fig. 1 Levels of radioactivity in the circulating haemolymph of crayfish at various times after injecting $\sim 5 \mu\text{g}$ radiiodinated BSA ($\square$), HGG ($\circ$) and crayfish haemocyanin ($\blacksquare$). Each experimental point represents the mean of duplicate counts on three animals. The vertical bars indicate the limits of ± 1 s.d.

The initial experiments were aimed at determining if the crayfish, *Procambarus clarkii*, could clear different proteins from the circulation. The basic protocol involved injecting into the heart of 25–30 g animals maintained at 23°C small amounts ($\sim 5 \mu\text{g}$ in 0.1 ml) of ^{125}I -labelled proteins¹¹. At various times samples of haemolymph were removed and assayed for radioactivity. Control (self) proteins included crayfish haemocyanin and a 5S crayfish protein of unknown function (purified by gel filtration from clotted haemolymph). Each of these self proteins, as depicted for crayfish haemocyanin in Fig. 1, seemed to equilibrate within 10 min (with a volume approximating 18% of the animal's weight) and to exhibit almost no elimination ($<20\%$) during 24 h. On the other hand (Fig. 1), if similar amounts of either iodinated bovine serum albumin (BSA) or human gamma globulin (HGG) were injected, relatively rapid clearance was observed. In the case of BSA about 80% of the radioactivity was eliminated within 2 h; elimination of HGG was somewhat slower. Two additional foreign proteins studied included keyhole limpet haemocyanin (KLH) and egg white lysozyme; the former was eliminated somewhat more slowly than HGG and the latter at about the same rate as BSA.

The next step was to determine the fate of these cleared proteins. First, several crayfish injected with BSA or HGG, maintained in small beakers containing ~ 5 ml water, did not excrete significant amounts of radioactivity during 24 h and thus the injected proteins were still within the animal. Second, as Table 1 shows, dissection of several animals 2 h after injection of BSA indicated that most of the cleared radioactivity was localised in the gills ($>60\%$ of that recovered). Further, the specific activity (counts per gram of tissue) of the green gland, an excretory organ, was also quite high. The relatively small amount of tissue present, however, suggests that this gland does not have a major role in the initial clearance.

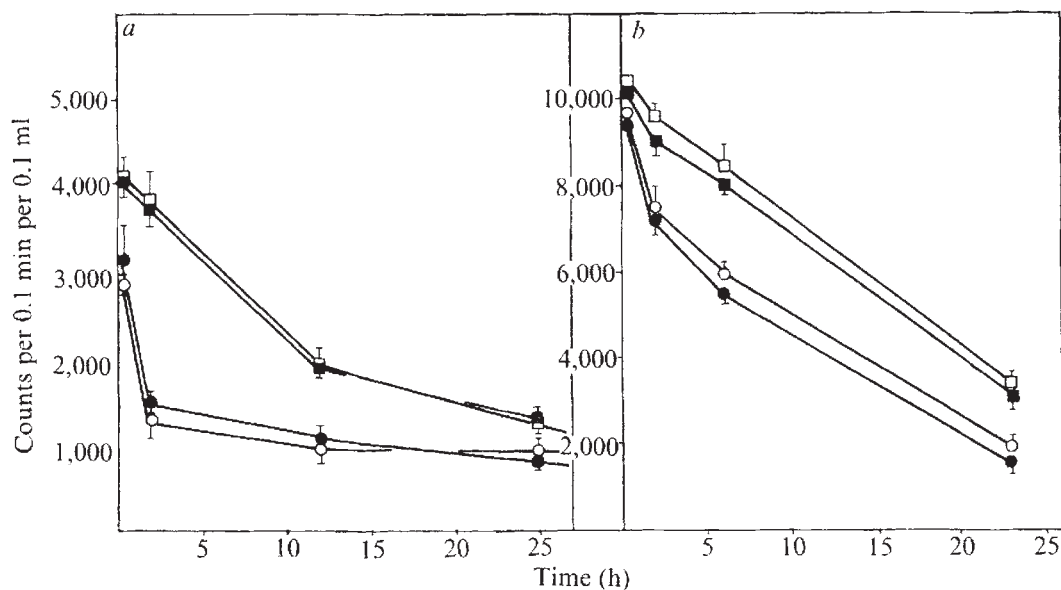


Fig. 2 Levels of radioactivity in the circulating haemolymph of crayfish at various times after injecting mixtures consisting of $\sim 5 \mu\text{g}$ of radiiodinated proteins and 5 mg unlabelled proteins. Each experimental point represents the mean of duplicate counts on three animals. The vertical bars indicate the limits of ± 1 s.d. a, ^{125}I -BSA plus; $\bullet$, nothing; $\circ$, bovine gamma globulin; $\square$, BSA; $\blacksquare$, human serum albumin. b, ^{125}I -HGG plus; $\square$, HGG; $\circ$, BSA; $\blacksquare$, BGG; $\bullet$, nothing.

Since the radioactivity injected on foreign protein was not cleared totally, trichloroacetic acid precipitation of the haemolymph was used to show if protein modification had occurred. The data obtained indicated that the 10-min BSA samples were ~ 95% precipitable, the 2-h samples were 90% precipitable, the 6-h samples ~ 70% and the 24-h samples only 50% precipitable. A similar pattern was obtained with HGG. On the other hand, the labelled circulating crayfish haemocyanin remained > 95% precipitable for 24 h. It therefore seems likely that the clearance curve for BSA shown in Fig. 1 represents two reactions—an initial concentration of the protein in the gills followed by degradation to small peptides which are released back into the circulation.

These experiments indicate that crayfish can readily discern self from non-self by clearing various foreign proteins from the circulation and concentrating them in the gills. We then investigated whether this recognition involved saturable receptors of reasonable specificity. We injected each animal with a mixture of ~ 5 µg ¹²⁵I-labelled protein and ~ 5 mg unlabelled protein. As Fig. 2a shows, excess unlabelled BSA seemed to retard the elimination of radioactive BSA whereas unlabelled BGG had no effect. Similarly KLH (not shown) had no effect on the elimination of ¹²⁵I-BSA whereas other serum albumins (human, shown in Fig. 2, and horse and rabbit, not shown), were as effective in retarding clearance as BSA when 5 mg was used. Alternatively, as Fig. 2b, shows both HGG and BGG slowed down the initial elimination of HGG whereas BSA and KLH (not shown) did not. Additional experiments (not shown) indicated that unlabelled KLH blocked the initial clearance of ¹²⁵I-KLH whereas BGG and BSA did not.

In conclusion the data reported here support the hypothesis that crayfish have naturally occurring receptors, or recognition molecules, for at least three groups of foreign proteins (albumins, gamma globulins and haemocyanins). Although it is premature to generalise about the significance and distribution of such putative receptors in other invertebrates, initial clearance and inhibition studies indicate their likely presence in another crustacean, the blue crab (McCumber and L.W.C., unpublished results) and in a mollusc, the chiton (Lafferty, presentation at Immunologic Phylogeny Symposium, Hawaii, June 1975). Optimistically, further pursuit of these studies might very well result "in the isolation of the primitive building block for vertebrate immunoglobulins"¹².

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aldehyde group of 11-*cis* retinal and an ε-amino group of L-lysine in opsin¹⁻⁴.

We report here the competitive inhibition of the combination of 11-*cis* retinal with opsin by β-ionone and discuss from this viewpoint the structure of the retinal-binding site of the opsin molecule.

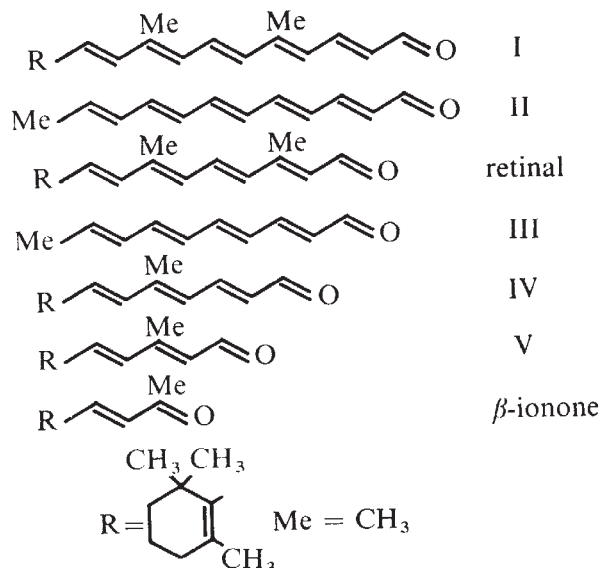
The existence of secondary interactions between 11-*cis* retinal and opsin has been suggested by many workers, without direct evidence. One possible way to study such interactions is to use 11-*cis* retinal analogues. Blatz *et al.*⁵ reported that the 11-*cis* retinal analogues shown in Fig. 1 do not form photosensitive pigments when incubated with cattle opsin. From their result one may conclude: (1) The length of the side chain of an 11-*cis* retinal analogue is a decisive factor in coupling with opsin. Retinal analogues having longer or shorter side chains than 11-*cis* retinal (compounds I, II, IV, and V) do not form photosensitive pigments. It may be said that opsin can discriminate between the lengths of the side chain of the retinal analogues. (2) A ring structure of the analogue seems essential for the regeneration of rhodopsin. Some analogues having a methyl group instead of a β-ionone ring (compounds II and III) fail to form a pigment.

We have now examined the possibility that the β-ionone ring of 11-*cis* retinal may interact with opsin through hydrophobic bonds; the regeneration of rhodopsin may begin with the formation of such bonds, and end in the formation of the protonated Schiff base linkage. Only when the aldehyde group of an analogue, already bound to opsin through the β-ionone ring, comes sterically near to the ε-amino group of an L-lysine residue at the 11-*cis* retinal-binding site in opsin (we call this the 'active L-lysine'), can the Schiff base be formed.

If that is so, we can expect that an analogue having a β-ionone ring, but a shorter side chain than retinal (compounds IV, V and β-ionone), may readily compete with 11-*cis* retinal in coupling with the site that anchors the ring structure.

Cattle opsin was prepared from ~ 50 light-adapted retinae by the usual methods⁶. The opsin was extracted with 2% digitonin in M/15 phosphate buffer (pH 6.5). Regeneration of rhodopsin occurred at 25 °C in an absorption cell of 1 cm light path placed in a recording spectrophotometer, and the absorbance at 498 nm was recorded as shown in Fig. 2a. Curve 1 shows the regeneration of rhodopsin from 11-*cis* retinal (final concentration 1.91×10^{-5} M) and opsin (final concentration 2.76×10^{-6} M). Addition of β-ionone (final concentration 3.68×10^{-4} M) to the reaction mixture slowed the regeneration of rhodopsin (curve 2). Both curves reach almost the same final

Fig. 1 Retinal and its analogues. None of these analogues form a photosensitive pigment on incubation with cattle opsin (Blatz *et al.*⁵).



Existence of a β-ionone ring-binding site in the rhodopsin molecule

RHODOPSIN is a carotenoid-protein, in which the 11-*cis* retinal chromophore, is coupled to the colourless protein, opsin. The linkage between them is a protonated Schiff base between the

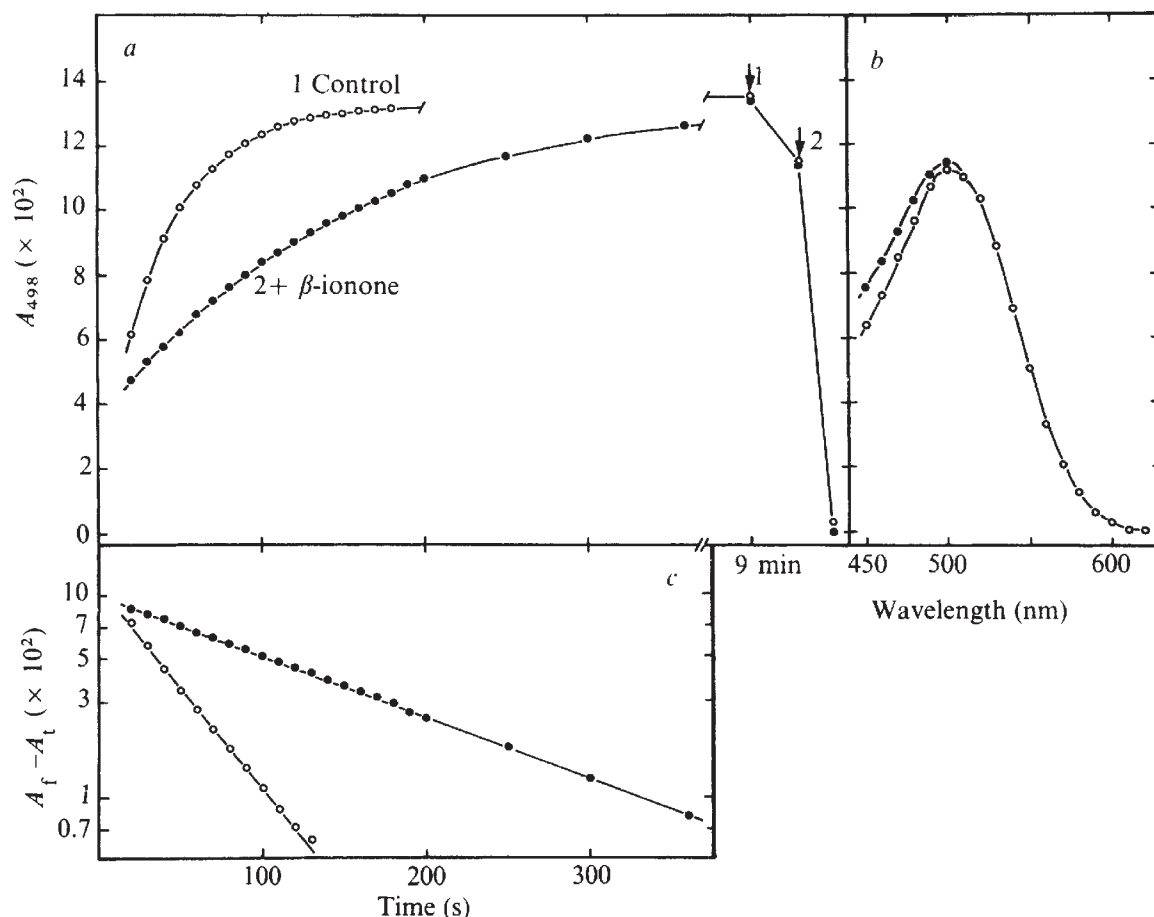


Fig. 2 Inhibition by β -ionone of the combination of 11-*cis* retinal with cattle opsin. *a*, Time course of the regeneration of rhodopsin. The reaction was performed at 25 °C in a cell (light path: 1 cm), containing 0.1 ml cattle opsin extracted with 2% digitonin (M/15 phosphate buffer pH 6.5), 2.0 ml M/15 phosphate buffer (pH 6.5) and 0.1 ml β -ionone (8.13×10^{-3} M) dissolved in 1% Brij 58 in M/15 phosphate buffer pH 6.5. In the control, 0.1 ml 1% Brij 58 was added instead of the β -ionone solution. The reaction was started by adding $10 \mu\text{l}$ 4.23×10^{-3} M 11-*cis* retinal in ethanol. The absorbance change at 498 nm was measured with a Hitachi 323 recording spectrophotometer. After the completion of the reaction $30 \mu\text{l}$ 1 M NH_4OH were added (arrow 1). Three minutes later, the preparations were irradiated with white light to bleach the regenerated rhodopsin (arrow 2). The concentrations of 11-*cis* retinal (ϵ_m : 24,900 in ethanol), cattle rhodopsin (ϵ_m : 40,600) and β -ionone (ϵ_m : 8,700 in ethanol) were determined spectrophotometrically. *b*, The difference spectra before and after bleaching of the regenerated rhodopsin. *c*, Pseudo first-order plots of Fig. 2*a*, where A_f is the final absorbance at 498 nm when the regeneration was completed and A_t is the absorbance at 498 nm at time, t .

levels of absorbance, indicating that no denaturation of opsin occurred in the experiment. After the completion of the regeneration reaction, hydroxylamine (final concentration 1.34×10^{-2} M) was added to both preparations (arrow 1 in Fig. 2*a*), and then the preparations were irradiated with white light to bleach the regenerated rhodopsin (arrow 2) completely. Figure 2*b* shows the difference spectra before and after bleaching. Because of the high absorbance caused by excess 11-*cis* retinal the spectra could not be measured correctly below 450 nm. Both difference spectra coincided reasonably with each other.

In this experiment both regeneration curves can be represented by pseudo first-order kinetics as shown in Fig. 2*c*, because there was 6.9 times as much 11-*cis* retinal as opsin. The pseudo first-order rate constants are $2.90 \times 10^{-2} \text{ s}^{-1}$ for regeneration in the absence and $7.45 \times 10^{-3} \text{ s}^{-1}$ in the presence of β -ionone. If one divides the pseudo first-order rate constants by the retinal concentration ($1.91 \times 10^{-5} \text{ M}$), the second-order rate constant of the regeneration in the absence or presence of β -ionone ($3.68 \times 10^{-4} \text{ M}$) is $1.52 \times 10^3 \text{ mol}^{-1} \text{ s}^{-1}$ or $3.94 \times 10^2 \text{ mol}^{-1} \text{ s}^{-1}$ respectively.

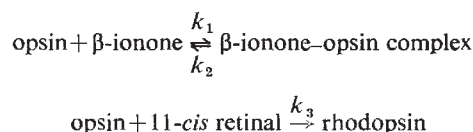
The regeneration rate constant of cattle rhodopsin was reported by Wald and Brown⁷ to be $43.3 \text{ mol}^{-1} \text{ s}^{-1}$ (22.5 °C, pH 7.0). The discrepancy between their rate constant and ours may be explained by the difference in digitonin concentration of the preparations—theirs being 2% and ours about 0.09%, and also by the presence of 0.045% Brij 58 in our experiments. We have found that the lower the concentration of digitonin, the greater the observed regeneration rate constant of cattle

rhodopsin, and that the presence of Brij 58 may slightly accelerate the regeneration (H.M. and T.Y., unpublished).

The possibility that the keto group of β -ionone may form a Schiff base with the active L-lysine of opsin was excluded by other experiments. Incubation of cattle opsin with β -ionone formed no pigment displaying absorbance between 300 and 700 nm. In addition, β -ionol, which was derived from β -ionone by reduction with sodium borohydride, also inhibits the regeneration of rhodopsin to the same extent as β -ionone. Thus, the inhibition by β -ionone must be attributed to its binding to some hydrophobic region of the retinal-binding site in opsin rather than to the active L-lysine. We call the hydrophobic region here the ' β -ionone ring-binding site'.

The binding of β -ionone to the β -ionone ring-binding site in the opsin molecule is faster than the formation of the Schiff base, because the addition of β -ionone at the beginning of the reaction does not change the regeneration kinetics of rhodopsin compared with when β -ionone is preincubated with opsin (H. M. and T. Y., unpublished).

One can write the mechanism as follows



where the rate constant of the formation of the β -ionone-opsin complex, k_1 , is much greater than that of rhodopsin formation,

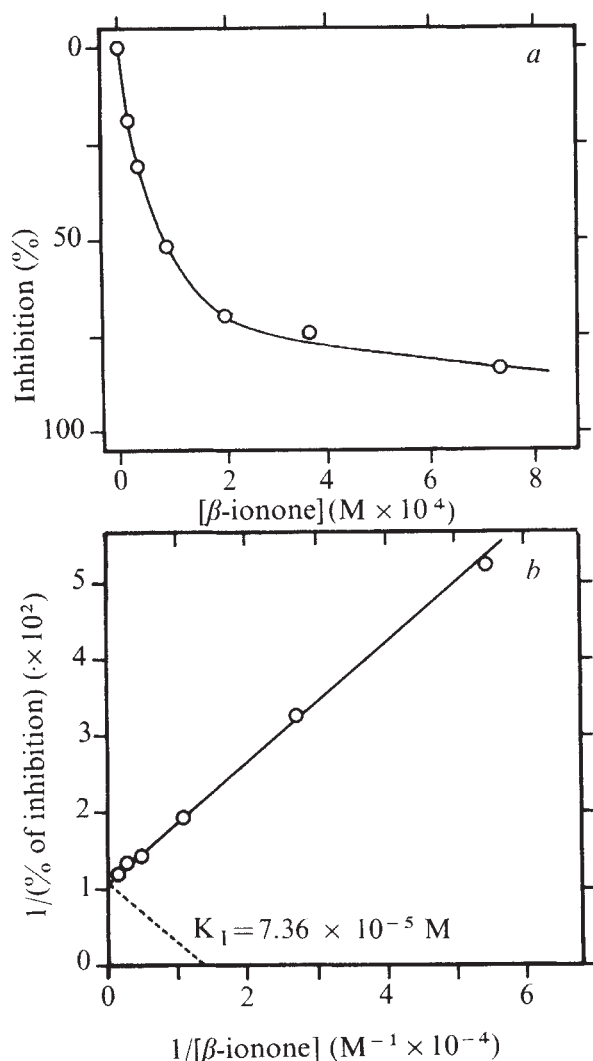


Fig. 3 Dependency of the inhibition on the concentration of β -ionone. *a*, Relationship between percentage inhibition and the concentration of β -ionone. The same reaction mixture were used as in Fig. 2 except that the volume of 1% Brij 58 solution was changed from 10 μ l to 0.2 ml. The total volume of the reaction mixture was adjusted to constant volume (2.2 ml) by addition or subtraction of M/15 phosphate buffer (pH 6.5). The percentage inhibition was calculated from the ratio of the observed pseudo first-order rate constants between the inhibited and the control reactions. *b*, Double reciprocal plot of Fig. 3a.

k_3 . So, one can determine the inhibition constant (K_I),

$$K_I = \frac{[\text{opsin}] [\beta\text{-ionone}]}{[\beta\text{-ionone-opsin}]} = \frac{k_2}{k_1}$$

by determining the relationship between the percentage of inhibition and the concentration of β -ionone (Fig. 3a) after measuring the saturation curve of the inhibitor, β -ionone. The curve of Fig. 3a has a hyperbolic shape and is described by the equation

$$\text{percentage of inhibition} = \frac{100 [\beta\text{-ionone}]}{K_I + [\beta\text{-ionone}]}$$

If one plots (percentage of inhibition)⁻¹ against (β -ionone)⁻¹, a straight line Lineweaver-Burk plot can be obtained as shown in Fig. 3b. The cross-over points at the abscissa or the ordinate show the K_I^{-1} or (maximum inhibition)⁻¹ respectively. The

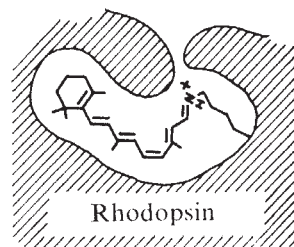


Fig. 4 A schematic representation of the structure of rhodopsin, in which the 11-*cis* retinal chromophore is covered by opsin (dashed area).

inhibition constant, K_I , for β -ionone is calculated to be $7.36 \times 10^{-5} \text{ M}$ and the maximum inhibition almost 100%.

Thus, the inhibition by β -ionone is competitive (not partially competitive) in the sense that β -ionone occupies the retinal-binding site so exclusively that the β -ionone-opsin complex is completely inactive in the regeneration reaction. The standard free energy change, ΔG° , of the formation of the β -ionone-opsin complex, calculated from the equation, $\Delta G^\circ = -RT \ln (1/K_I)$, was $-5.6 \text{ kcalorie mol}^{-1}$.

We illustrate a hypothetical configuration of rhodopsin in Fig. 4. The chromophore in the figure is drawn as 6-*s-cis*, 11-*cis*, 12-*s-cis* according to the recent X-ray analysis of 11-*cis* retinal⁸. The β -ionone ring of 11-*cis* retinal is postulated to be bound to the β -ionone ring-binding site in opsin. The fact that rhodopsin is not attacked by hydroxylamine⁹ and sodium borohydride¹⁰ (which do attack chicken iodopsin^{9,11}) indicates that the Schiff base linkage may be covered by opsin. The polyene side chain of the retinal chromophore is also covered, because lipoxidase does not oxidise the chromophore of cattle¹² and chicken¹¹ rhodopsin.

According to our model, only those retinal analogues which have the same length of side chain and structure of the β -ionone ring as retinal (as, for example, 9-desmethylretinal, 13-desmethylretinal and 14-methylretinal) are expected to form photosensitive pigments by binding with opsin. This, in fact, has been shown to be the case^{5,13,15,16}.

The pocket of the β -ionone ring-binding site is thought to have a relatively low stereospecificity, since α -ionone also inhibits the regeneration of rhodopsin competitively (H. M. and T. Y., unpublished). Also, 5-vitaldehyde¹⁴, which has a five-membered ring in place of the β -ionone ring of retinal, and other analogues of retinal which have slightly modified β -ionone rings such as 5,6-dihydroretinal^{5,15}, 4-oxoretinal¹⁷, 5,6-monoperoxiretinal¹⁸, 3-dehydroretinal¹⁹, α -retinal²⁰ and 5,6-epoxy, 3-dehydroretinal²¹, form photosensitive pigments.

Wald²² pointed out that the retinal chromophore in visual pigments can fit in a pocket of the surface structure of opsin in two possible ways—by weak bonding through hydrogen bonds or van der Waals' forces, and so-called caging or clathrate formation, in which the chromophore is simply surrounded by opsin without any interacting forces. The low degree of stereospecificity of opsin to the ring structure of the chromophore may support the latter mechanism. To confirm this viewpoint, more detailed kinetic studies on the inhibition of rhodopsin synthesis by β -ionone and other retinal analogues are in progress in our laboratory.

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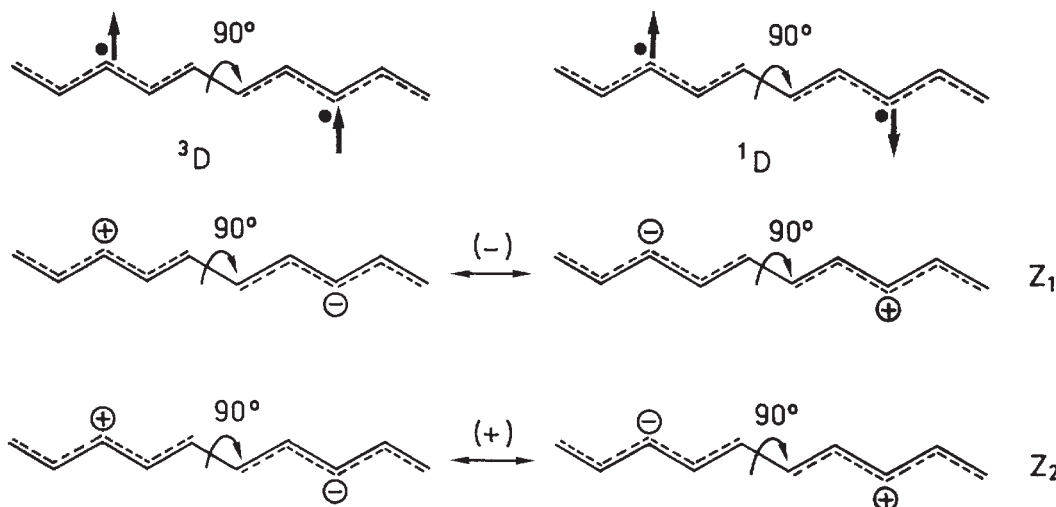
Conversion of a photon to an electrical signal by sudden polarisation in the N-retinylidene visual chromophore

ONE of the major unresolved problems in the chemistry of vision remains the detailed mechanism by which absorption of a photon by rhodopsin can ultimately change the permeability of the rod plasma membrane to Na^+ ions¹. We demonstrate here that excitation to the lowest singly-excited π, π^* singlet state of a realistic model of the visual chromophore triggers a sudden, short-lived, electrical signal in which charge moves from one end of the molecule to the other. We suggest that this electrical signal is the crucial, primary event in the overall mechanism leading to vision.

Diradical intermediates have four chemically important electronic states², the properties of which form an integral part of the theory of photochemical reactions²⁻⁵. We can illustrate the nature of these four states on the orthogonal di-(all-*trans*) pentadienyl diradical, formed by a 90° twist about the central C-C bond of all-*trans* decapentaene



The two lowest states are a (triplet, singlet) pair of diradical states ^3D and ^1D , and above are two singlet zwitterionic states



Z_1 and Z_2 (Fig. 1). These are correctly represented by out-of-phase (-) and in-phase (+) combinations of the two ionic resonance structures.

It has been shown⁶, in confirmation of an earlier prediction⁷, that the lowest excited Z_1 zwitterionic states of organic diradicals are strongly polarised if a slight dissymmetry exists between the two odd-electron sites. A sudden, large separation of charge occurs. This 'sudden polarisation effect'⁶ can be introduced by a molecular distortion, substitution, or better still by strong perturbations such as the replacement of a carbon atom by a heteroatom. For instance, we would expect the Z_1 excited states of the Schiff base and the protonated Schiff base of dipentadienyl to be strongly polarised (Fig. 2). In both systems the negative charge will flow into the fragment which carries the nitrogen atom. In the Schiff base, the nitrogen will carry most of the negative charge, whereas in the protonated Schiff base the incoming negative charge cancels the positive charge on the nitrogen atom, leaving a now neutral amino group, and a second, newly positively charged, pentadienyl moiety. As the primary photoprocess in rhodopsin is known¹ to involve the isomerisation of the 11-*cis* N-retinylidene chromophore—possibly in the 12-*s-cis* conformation⁸ and most probably as a protonated Schiff base⁹—we have investigated the charge distribution in the excited singlet state of the 11-*orthogonal* protonated Schiff base of the retinal skeleton, as well as of the Schiff base itself.

As a realistic model of the chromophore we used molecule I (nonatetraenylidene-methyliminium ion), initially in the 11-*cis*, 12-*s-cis* conformation (for convenience, we number the atoms as in the full retinylidene skeleton). The C5=C6 bond of the cyclohexene ring, which is out of conjugation with the 7-11

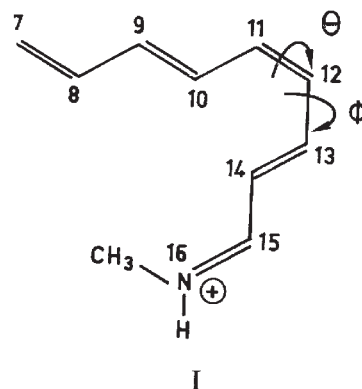


Table 1 Energies and charge distributions in 11-twisted, 12-*s-cis* protonated Schiff base I

Twist angle	0°	30°	60°	80°	90°	
Relative energies (eV)						
Ground singlet	0.	-0.23	0.73	1.11	1.18	(¹ D)
Excited singlet	4.66	4.57	4.04	3.48	3.39	(Z ₁)
Net charge in 7-11 fragment:						
Ground singlet	+0.17	+0.19	+0.23	+0.23	+0.23	(¹ D)
Excited singlet	+0.41	+0.40	+0.55	+0.98	+1.11	(Z ₁)
Net charge in 12-16 fragment:						
Ground singlet	+0.83	+0.81	+0.77	+0.77	+0.77	(¹ D)
Excited singlet	+0.59	+0.60	+0.45	+0.02	-0.11	(Z ₁)

Bond lengths: all CH = 1.08 Å, except CH = 1.09 Å in CH₃. At 0° and 30°, C=C = 1.34 Å, C-C = 1.47 Å. For 60°, 80° and 90°, all C-C = 1.40 Å except 11-12 = 1.50 Å. Finally, C=N = 1.31 Å, NH = 1.02 Å. The open-shell Hamiltonian is used for the excited singlet throughout and for the ground singlet from 60-90°. The closed-shell Hamiltonian is used for the ground singlet at 0 and 30°.

program (W. Hehre, personal communication), using a minimal basis set of orbitals and completed by a three-by-three configuration interaction treatment for the proper description of diradicals. Both closed-shell and restricted open-shell Hamiltonians can be used to calculate the energy.

We have calculated energies and charge distributions of the ground and lowest singly-excited π, π^* singlet states of I for various values of the 11-12 twist angle θ (Table 1). We used the experimental¹⁰ torsional angle ϕ about the 12-13 bond at $\theta = 0^\circ$, and interpolated linearly from $\phi = 39^\circ$ to $\phi = 0^\circ$ as θ increases from 0-90°. Table 1 shows that, contrary to the nearly constant charge distribution in the ground ¹D diradical state, there is a

starting molecule to Z₁ by assuming a negative counterion fixed in the vicinity of the initially charged nitrogen atom. Then the initial dipole moment $\mu \approx 0$ and the change in dipole moment $\Delta\mu$ on excitation and twist to the 11-*orthogonal* 12-*s-cis* form is given by $\mu(Z_1)$. Since atoms 9 and 16 are separated by ~ 6.8 Å in this conformation, $\Delta\mu \approx 33$ Debyes. We have verified that the charge polarisation occurs also in the 12-*s-trans* form. In this case, however, the C9-N16 distance in the *orthogonal* conformer is ~ 8.3 Å and the concomitant dipole moment change $\Delta\mu \approx 40$ Debyes.

The vertical excitation energy calculated for I is too high (4.66 eV compared with the experimental value of 2.75 eV,

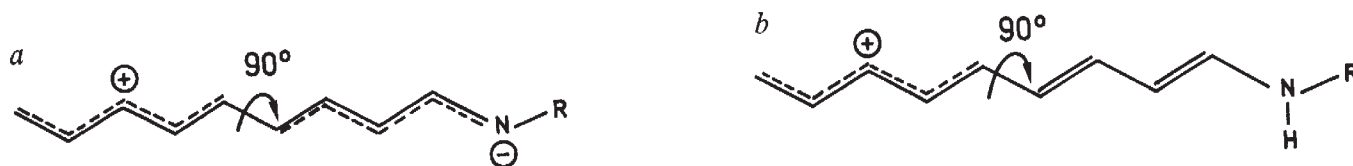


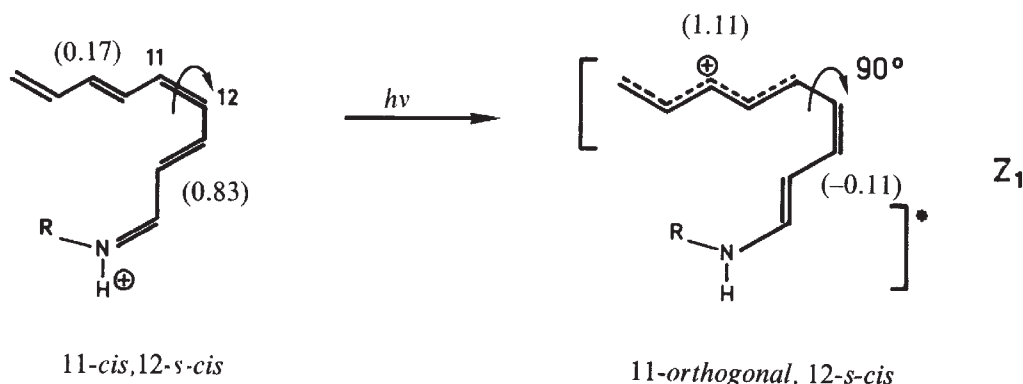
Fig. 2 Expected polarisation of excited Z₁ states in unsymmetrical polyenes: a, Schiff base; b, protonated Schiff base.

sharp polarisation of the excited π, π^* state as the twist angle reaches 80°. The positive charge, which is on the nitrogen grouping in the coplanar ground state, shifts somewhat towards the 7-11 moiety in the vertically-excited state, which has considerable intramolecular charge transfer characteristics. The greatest change occurs, however, as this excited singlet molecule twists from 60° to the fully *orthogonal* conformation. All the positive charge flows into the 7-11 pentadienyl fragment, with even a slight negative residual charge left on the Schiff base site (Fig. 3).

The positive charge in excited Z₁ is evenly distributed over atoms 7, 9 and 11, where the amplitude of the non-bonding molecular orbital of the 7-11 pentadienyl fragment is large. This leads to the migration, on excitation, of the positive charge from atom 16 to an average position at atom 9, that is, over a length of seven bonds. The net effect is an extremely large electrical signal. We can estimate the change in dipole from

ref. 11). Single-configuration, minimal basis calculations are notoriously poor for vertical π, π^* singlet-state excitation energies¹². The restricted open-shell method used here, however, is particularly appropriate for describing the 90°-twisted molecule, as the two 'non-bonding' π orbitals Ψ_5 and Ψ_6 are then well separated from the remaining π manifold. The three-by-three configuration interaction is a further guarantee that the four lowest-lying states are correctly described³. In this respect the calculated barrier to isomerisation in the ground singlet state (27.2 kcalorie mol⁻¹) agrees nicely with experimental results (25.5 kcalorie mol⁻¹, ref. 13). Finally, the deep calculated minimum (1.27 eV) in the excited twisted singlet is in contradiction with the previous PPP pure π -electron calculations^{14,15}. The downwards slope may be emphasised by the exaggerated vertical excitation energy, but the qualitative behaviour seems reasonable since the twisting—just as in ethylene—enables a π^* orbital, which is 11-12 antibonding, to become non-bonding.

Fig. 3 Excitation and partial isomerisation of the protonated Schiff base (net charges in parentheses).



The previous calculations, with only single excitations^{14,15}, possibly suffer from an inappropriate description of the wavefunction for Z_1 , which involves a double excitation ($\lambda\Psi_5^2 - \mu\Psi_6^2$).

Clearly, the complete retinal system was not included in the calculation and no external effect was considered. Although we have not been able to investigate the skeleton containing the additional C5–C6 and C6–C7 bonds, or cyclohexene ring, we feel that the qualitative outcome must certainly be the same. Within the ion pair created in the twisted Z_1 state the negative charge must inexorably be attracted by the pre-existing positive charge on the Schiff base site. We have verified that the sudden polarisation effect also occurs in the unprotonated Schiff base of I, where it is accompanied by a significant increase in the basicity of the nitrogen atom. If rhodopsin were to involve a weak $N \cdots H$ bond (ref. 16 and M. Ottolenghi, personal communication), the polarisation effect could trigger a displacement of the proton toward the nitrogen atom (in I itself, we find no evidence for a tendency to lose the Schiff base proton, contrary to a recent suggestion¹⁷). We also note that some experimental evidence for a strongly polarised twisted singlet excited state does exist in retinol¹⁸.

We suggest that the electrical signal revealed here occurs as the crucial, primary event in the photoprocess leading to vision. A crude classical estimate of the time required for the rotation of a rigid rotor, with moment of inertia equivalent to that of retinal, shows that the duration of such a signal is consistent with the 6 ps required for formation of prelumirhodopsin¹⁹. The electrical signal can act as a trigger on the medium, as the Z_1 state has 1–2 eV excitation energy which can be used as a source of energy to influence the system. There may be either a direct, electrostatic interaction on neighbouring 'pores', which could affect the disk membrane permeability to Na^+ ions, or an indirect interaction by way of induction of a conformational change in protein.

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cerebellar ataxia, deafness, mental retardation or other abnormalities. Some examples of such syndromes are Batten-Spielmeyer-Vogt disease, Refsum's disease, Laurence-Moon Biedl syndrome, Bassen-Kornzweig disease, Usher's syndrome and several of the mucopolysaccharidoses^{1–4}. As far as we know laboratory animals with neurological syndromes that include photoreceptor degeneration have not been available.

We describe here two types of slow, progressive photoreceptor degeneration that have been discovered in two neurological mutant mice that lose cerebellar Purkinje cells. The first, Purkinje cell degeneration, gene symbol *pcd*, is a new autosomal recessive mutation that results in loss of virtually all Purkinje cells between the ages of 3 and 5 weeks⁵. The second is the nervous, *nr*, mutant which also loses Purkinje cells relatively early but to a lesser extent; up to 90% of the Purkinje cells in *nr/nr* mice degenerate between 3 and 7 weeks of age^{5,6}.

The *pcd* mutation occurred in the C57BR/cdJ strain but is now being transferred⁹ to C57BL/6. The *nr* mutation occurred in the BALB/cGr strain and is coisogenic with that strain^{5,6}. These two mutations are not linked, nor are they alleles of or linked to the retinal degeneration, *rd*, gene⁹.

Homozygous mutants were identified by their ataxia resulting from the cerebellar defect at ages older than 15 and 22 d for *nr/nr* and *pcd/pcd*, respectively. Younger mutants and controls were identified by the cytological appearance of cerebellar Purkinje cells. Mice were reared in cyclic light⁷. Eyes of mutants and normal littermates at several ages were enucleated, fixed, embedded in plastic and sectioned as described elsewhere⁷.

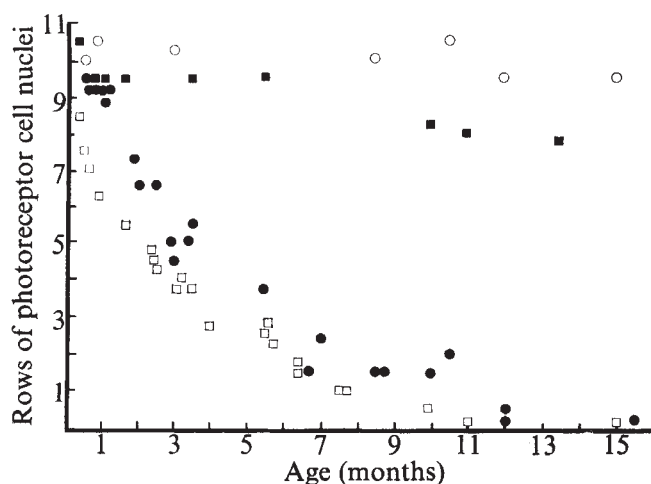


Fig. 1 Thickness of the outer nuclear layer measured as rows of photoreceptor nuclei at the posterior pole of the eye. Each point represents one animal. The number of rows of photoreceptor cell nuclei at the posterior pole of the eye (400–800 μ m from the optic nerve head) was recorded as a range (for example, 4–5.5 rows), and the mid-point of the range was plotted. For both *pcd/pcd* and *nr/nr*, the average range was 1.5 rows. The cell loss in nervous controls may be an effect of albinism, light, heterozygosity for *nr* (oldest controls were known +/*nr*) or some interaction among these factors. $\square$, *nr/nr*; $\blacksquare$, +/*nr* or +/+; $\bullet$, *pcd/pcd*; $\circ$, +/*pcd* or +/+.

The time course of the retinal disease is similar in the two mutants although the loss of photoreceptor cells begins earlier and proceeds slightly faster in *nr/nr* than in *pcd/pcd* mutants (Fig. 1). Twenty-five to thirty per cent of the cells disappear by 3 weeks in *nr/nr*, but not until 2 months of age in *pcd/pcd*.

Retinae of *pcd/pcd* mice seem relatively normal at day 18, although there may be a slight increase over controls in the number of pyknotic photoreceptor cell nuclei. By day 25, however, there is an obvious increase in the number of pyknotic nuclei, and the rod outer segments are clearly disorganised (Fig. 2a; compare Fig. 2h). As the loss of photoreceptor cells

Two new types of retinal degeneration in cerebellar mutant mice

HUMAN retinitis pigmentosa represents a class of diseases, the principal characteristic of which is the slow and progressive degeneration of photoreceptor cells. In several human neurological syndromes, photoreceptor degeneration accompanies

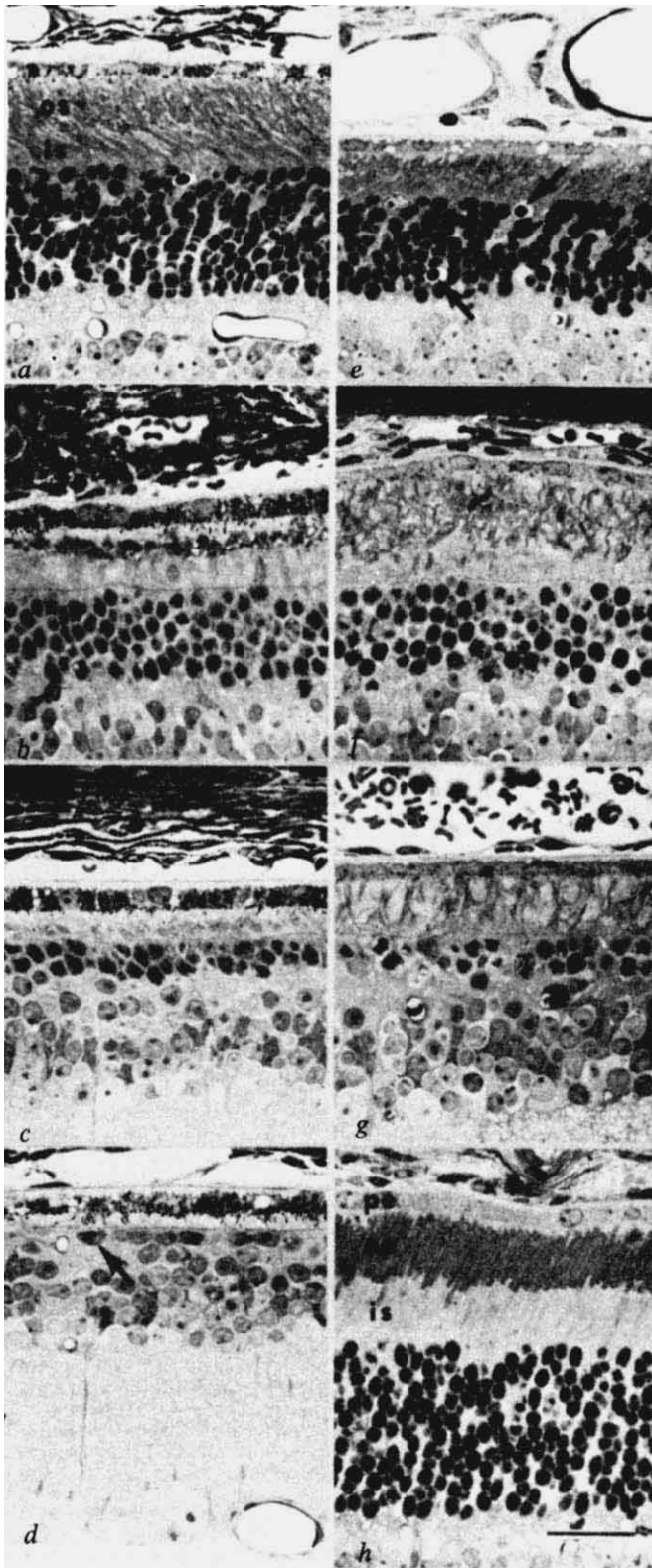


Fig. 2 Photomicrographs of retinæ from mice of different ages and genotypes. *a-d*, *pcd/pcd*; *e-g*, *nr/nr*; *h*, normal (+/*nr* or +/+). *a*, 25 d of age; *b*, 5.5 months; *c*, 6.5 months; *d*, 15.5 months; *e*, 19 d; *f*, 3 months; *g*, 6.5 months; *h*, 2.5 months. Several pyknotic nuclei are evident in the outer nuclear layer of the *nr/nr* retina at 19 d of age (*e*, arrows), and the outer nuclear layer is thinner than that in normal retinæ (*h*) or that in *pcd/pcd* mutant retinæ of slightly older age (*a*). The outer segment layer is thicker in *nr/nr* retinæ than in *pcd/pcd* retinæ at stages of comparable photoreceptor cell loss (compare *f* with *b* and *g* with *c*). A residual photoreceptor cell is present after the rest of the outer nuclear layer has disappeared (*d*, arrow). The inner retinal layers appear relatively normal after photoreceptor degeneration, at least up to 16 months of age (*d*), is, inner segments; os, outer segments; pe, pigment epithelium. 1–2 μ m Epon–Araldite sections. Toluidine blue. All magnifications the same; the bar represents 25 μ m.

continues (Fig. 2*b-d*), the outer segments become shorter and more variable in length. There are regional differences in rate of photoreceptor degeneration in *pcd/pcd* similar to those observed in pigmented rats with inherited retinal dystrophy⁷.

The retinal pathology of the nervous mutant is different. The youngest *nr/nr* animal we have examined was 13 d old, yet there are already many pyknotic nuclei, and the rod outer segments are disorganised and slightly shorter than those of littermate controls. These features are more conspicuous at day 19 (Fig. 2*e*). A few large whorls of outer segment membranes may be present as early as 3 weeks of age. These whorls accumulate so that by 2.5–3 months, the outer segment zone is comprised almost exclusively of whorls with few distinct outer segments (Fig. 2*f*). As a consequence of this accumulation, the outer segment zone in *nr/nr* mice is thicker than that in *pcd/pcd* mice with comparable cell loss (compare Fig. 2*b* and *f* with *c* and *g*) although the outer segments in *pcd/pcd* are usually more distinct. The outer segment zone in *nr/nr* becomes thinner after 3–4 months of age as additional photoreceptors are lost, and by 11 months no outer segment membranes are present. The membranous whorls in *nr/nr* resemble those seen in rats with inherited retinal dystrophy⁷. In a preliminary electron microscopic examination of *nr/nr* mice of several ages, mitochondria in photoreceptor inner segments were found to be larger in cross section than those of *pcd/pcd* or of controls; similarly, rounded mitochondria are an early feature in cerebellar Purkinje cells of *nr/nr* mice⁶. In *nr/nr* retinæ the regional differences in the rate of photoreceptor degeneration are less striking than in *pcd/pcd*.

In both the *pcd/pcd* and *nr/nr* mice, photoreceptor degeneration consistently accompanied cerebellar ataxia. The 28 *pcd/pcd* and 29 *nr/nr* animals exhibiting ataxia had the appropriate type of retinal degeneration, and the 12 controls for *pcd/pcd* (+/*pcd* and +/–) and 34 controls for *nr/nr* (+/*nr* and +/–) had normal retinæ. The retinæ of other adult mutants with different cerebellar defects (*reeler*, *weaver*, *staggerer* and *leaner*) were examined; all were histologically normal.

The cerebellar disease in both *pcd/pcd* and *nr/nr* becomes evident about 2 weeks after birth and is virtually complete within a few weeks. Apart from this temporal similarity, however, the Purkinje cell lesions in the two mutants are cytologically distinct⁹. Retinal abnormality in both is first evident at about 2–3 weeks of age, but unlike the precipitous degeneration of cerebellar Purkinje cells, the visual cells disappear slowly during a year. It is obvious, however, that the retinal diseases of the two mutants are also distinct. The most notable differences are (1) the earlier onset in *nr/nr*; (2) the failure of *nr/nr* to develop rod outer segments at a normal rate; (3) the persistence of short, yet distinct rod outer segments in *pcd/pcd* compared with the accumulation of whorls of outer segment membranes in *nr/nr*; (4) the presence of enlarged mitochondria in *nr/nr* inner segments; and (5) the regional differences in rate of degeneration in *pcd/pcd* mutants. The significance of the differences in rate and distribution of the photoreceptor degeneration in *pcd/pcd* and *nr/nr* mutants is difficult to assess because the mutations are on different genetic backgrounds. The fact that *pcd* and *nr* are on pigmented and albino backgrounds respectively, is perhaps an important factor, since in rats with inherited retinal dystrophy the rate of degeneration is influenced by the amount of light to which the retina is exposed, either environmentally or as a result of differences in eye pigmentation⁷.

Thus, *pcd/pcd* and *nr/nr* mutant mice exhibit two different types of retinal degeneration, similar in rate but probably different in aetiology. Unlike the rapid degeneration of photoreceptor cells in the *rd/rd* mutant mouse⁸, these new retinal degenerations have a slow progression resembling that seen in the retinitis pigmentosa class of diseases in man. Analysis of the *pcd/pcd* and *nr/nr* mutants hopefully will provide insight into inherited retinal degenerations and their relationship to inherited degenerations of other neuronal classes in the central nervous system.

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Effect of denervation and local damage on extrajunctional L-glutamate receptors in locust muscle

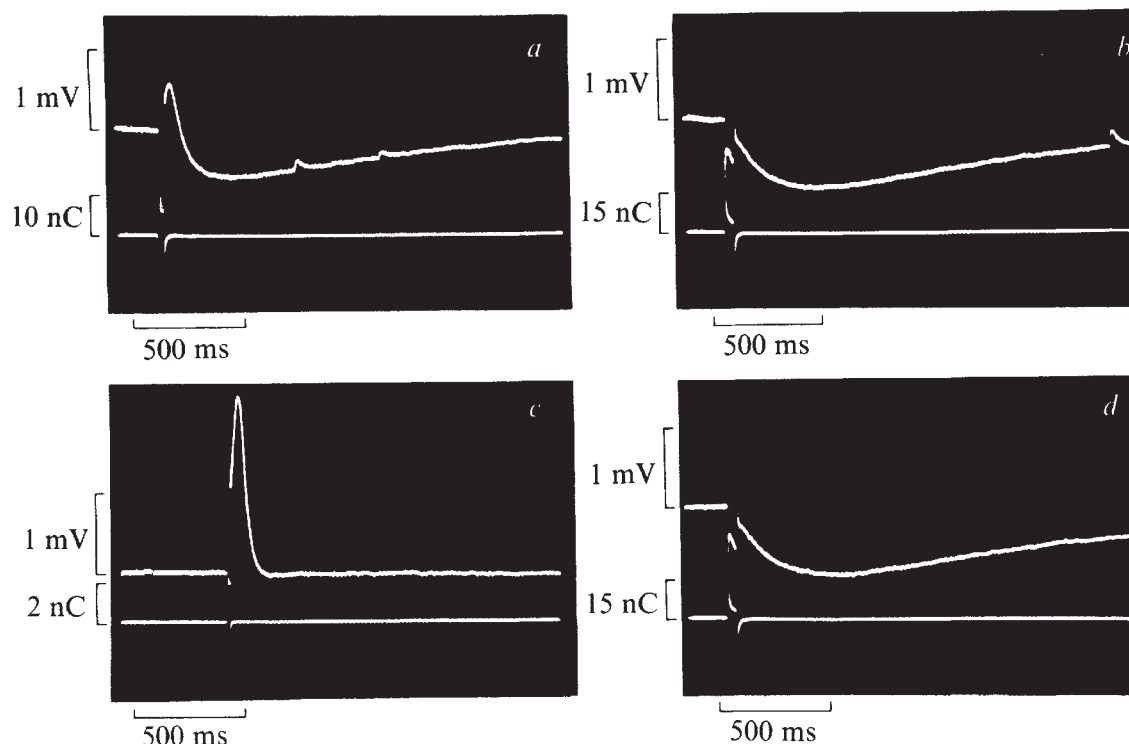
EXTRAJUNCTIONAL receptors occur in both normal vertebrate muscle¹ and locust muscle² as demonstrated by iontophoretically applied acetylcholine (ACh) and L-glutamate respectively. Two types of extrajunctional glutamate receptors occur in locust muscle in addition to the junctional receptors³. The biphasic response (depolarisation preceding hyperpolarisation) seen when glutamate is applied iontophoretically to extrajunctional regions of locust muscle results from simultaneous activation of the

two types of extrajunctional glutamate receptors³. The D receptor (which mediates a depolarisation) is activated by L-glutamate but insensitive to the glutamate analogue DL-ibotenate, while the H receptor (which mediates a hyperpolarisation) is activated by both L-glutamate and DL-ibotenate. This pharmacological difference has allowed separate investigation of the two types of receptors.

Following denervation vertebrate muscle develops a high degree of extrajunctional sensitivity to ACh^{4,5}, whereas locust muscle develops a high degree of extrajunctional sensitivity to glutamate⁶. The transection of vertebrate muscle causes the development of extrajunctional sensitivity to ACh at the site of damage⁷. It was therefore of interest to see whether both types of extrajunctional receptors normally present in locust muscle are involved in the changes in sensitivity of the extrajunctional membrane after denervation, and to determine if similar changes occur after muscle damage.

Extrajunctional glutamate receptors were investigated in the extensor tibiae muscle of adult locusts (*Schistocerca gregaria*). Receptors in normal muscles were compared with those in muscles which were either denervated by transection of the nerve supply⁸ or were damaged locally with a single scalpel cut. The sensitivities of extrajunctional receptors were mapped using double-barrel micropipettes of high resistance^{3,9} (150-300 MΩ) which contained L-glutamate (sodium salt, 0.1 M, pH 8) in one barrel and the glutamate analogue DL-ibotenate¹⁰ (sodium salt, 0.1 M, pH 7) in the other. Micropipettes were moved in 10 or 20 μm steps across muscle fibres (~100 μm wide), or along sections of the outer faces of the fibres. Glutamate, which activates both types of receptors, was used to measure D-receptor sensitivity in situations where the D response was the predominant one. H-receptor sensitivity was obtained with ibotenate, which activates only H receptors. In situations where the sensitivity of the D receptors was low, the H receptors were first desensitised with ibotenate so that glutamate now activated only the D receptors to give an accurate value for their sensitivity. As the H response had an equilibrium potential which lay close to the resting potential of the muscle fibres, care was taken that

Fig. 1 The effect of denervation on the extrajunctional response to iontophoretically applied glutamate and ibotenate, applied from adjacent barrels of a high resistance micropipette. Drug ejection currents passing through the micropipette are monitored on the lower traces. *a*, Control muscle, extrajunctional response to glutamate; *b*, control muscle, extrajunctional response to ibotenate; *c*, denervated muscle, extrajunctional response to glutamate; *d*, denervated muscle, extrajunctional response to ibotenate.



control muscle, extrajunctional response to glutamate; *b*, control muscle, extrajunctional response to ibotenate; *c*, denervated muscle, extrajunctional response to glutamate; *d*, denervated muscle, extrajunctional response to ibotenate. Note that a smaller quantity of glutamate than was used in *a* produces a greatly increased D response. The quantity of glutamate used is insufficient to produce a significant H component; *d*, denervated muscle, extrajunctional response to ibotenate; a single component H response is present. Miniature excitatory potentials are seen on the traces before denervation.

this did not introduce error in sensitivity measurements.

Both denervation and local damage of the muscle fibres resulted in increased extrajunctional sensitivity to glutamate in animals kept at room temperature for 10 to 14 d. Following denervation, the entire extrajunctional membrane developed a uniform increased sensitivity to glutamate. Thus the iontophoretic application of glutamate, to denervated muscle fibres, in quantities smaller than those normally needed to produce an extrajunctional response in innervated muscle, resulted in a depolarisation with a short latency (Fig. 1). If the quantity of glutamate applied to the denervated muscle was as large as that normally used to produce an extrajunctional response in innervated muscle, a biphasic response was obtained, although the H component was partly masked by the large D component. The iontophoretic application of ibotenate demonstrated the presence of the H component in denervated muscle fibres. As the increased sensitivity initially developed in a non-uniform manner, it was possible to compare the relative sensitivities of the two types of extrajunctional receptors in regions of the same muscle fibre which differed in sensitivity. In such situations, although the D-receptor sensitivity varied severalfold in different regions, the H receptors demonstrated no significant change and gave values which fell within the ranges encountered in normal control muscles (see Fig. 2).

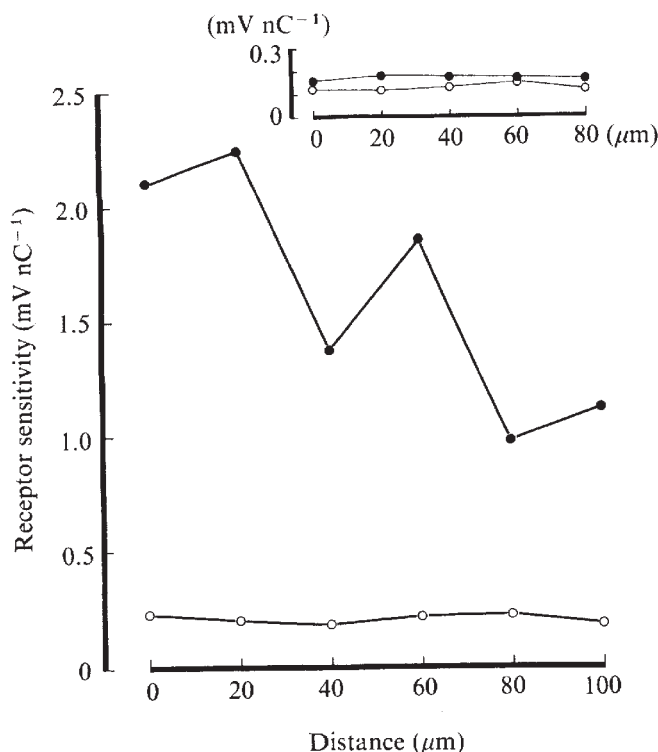


Fig. 2 Graph of the distribution of extrajunctional receptor sensitivity in a muscle fibre after denervation. Inset shows the sensitivity of extrajunctional glutamate receptors in a typical innervated fibre from a control muscle (80 μm wide). ●, D-receptor sensitivity to glutamate. ○, H-receptor sensitivity to ibotenate. Abscissa, distance across the muscle fibre surface.

Locally damaged fibres⁷ developed an increased extrajunctional sensitivity to glutamate which was highest at the site of local damage and fell to normal levels within $\sim 100 \mu\text{m}$. The H receptors demonstrated no measurable change in sensitivity in different regions of the same fibre, giving values similar to control muscles, whereas the D receptors changed by approximately 100-fold from 5 mV nC^{-1} at the site of injury to $50 \mu\text{V nC}^{-1}$ in other parts of the same fibre.

The increased sensitivity involved receptors which

mediated ionic permeability changes to Na^+/K^+ . In this respect they resemble the extrajunctional D receptors in innervated muscle². The H responses produced by ibotenate, applied iontophoretically to extrajunctional regions of high glutamate sensitivity, had equilibrium potentials similar to those obtained in normal control muscles indicating increased Cl^- permeability³.

Thus, when extrajunctional sensitivity to glutamate was altered by two different methods, that is, denervation and mechanical damage, the high degree of sensitivity which developed was the result of an increased sensitivity of the D receptors with no apparent change in H receptors. This, and the observation that, following denervation and muscle damage, D receptors varied considerably in sensitivity in different parts of the same fibre while the sensitivity of the H receptors remained unaltered, demonstrates that the two types of extrajunctional receptors can change independently.

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Relationship of spontaneous fibrillation potentials to muscle fibre segmentation in human muscular dystrophy

DENERVATED skeletal muscle fibres have long been known to show spontaneous fibrillation potentials, this being one of the features resulting from the removal of the trophic influence of the motor nerve¹. Electromyography (EMG) has shown spontaneous fibrillations in human myopathies such as myositis or muscular dystrophy^{2–6}, but their true incidence and mechanism is still obscure. We propose that myopathic fibrillations result from segmental necrosis of muscle fibres so that a distal fibre segment is separated from the part carrying the motor endplate. We show here, first, that after experimental myotomy in the baboon biceps muscle, the nerve-free segments develop fibrillation after a consistent delay and second, that by comparing different clinical types of human myopathies a correlation can be found between the incidence of focal necrosis and spontaneous fibrillation potentials. Our findings are consistent with our previous report of collateral innervation of newly formed muscle fibres in Duchenne muscular dystrophy⁷.

Experimental myotomies were performed in sterile conditions in seven healthy adult baboons or macaques, under light pentothal anaesthesia. EMG tests and biopsies were then carried out and the animals were not killed. The biceps brachii muscle was used because its motor endplates are concentrated in a narrow innervation zone⁸ which can be identified on the exposed muscle by focal stimulation with a sterile steel electrode placed on the superficial muscle fibres. Threshold current pulses of 0.1 ms duration (about 1 mA) elicited brisk twitches at the motor point but the same stimuli were ineffective when sited more distally on the same bundles (Fig. 1a). A number of small cuts were made in several muscle bundles with iridectomy scissors, at least 10–15 mm distal to the motor point thus defined. We

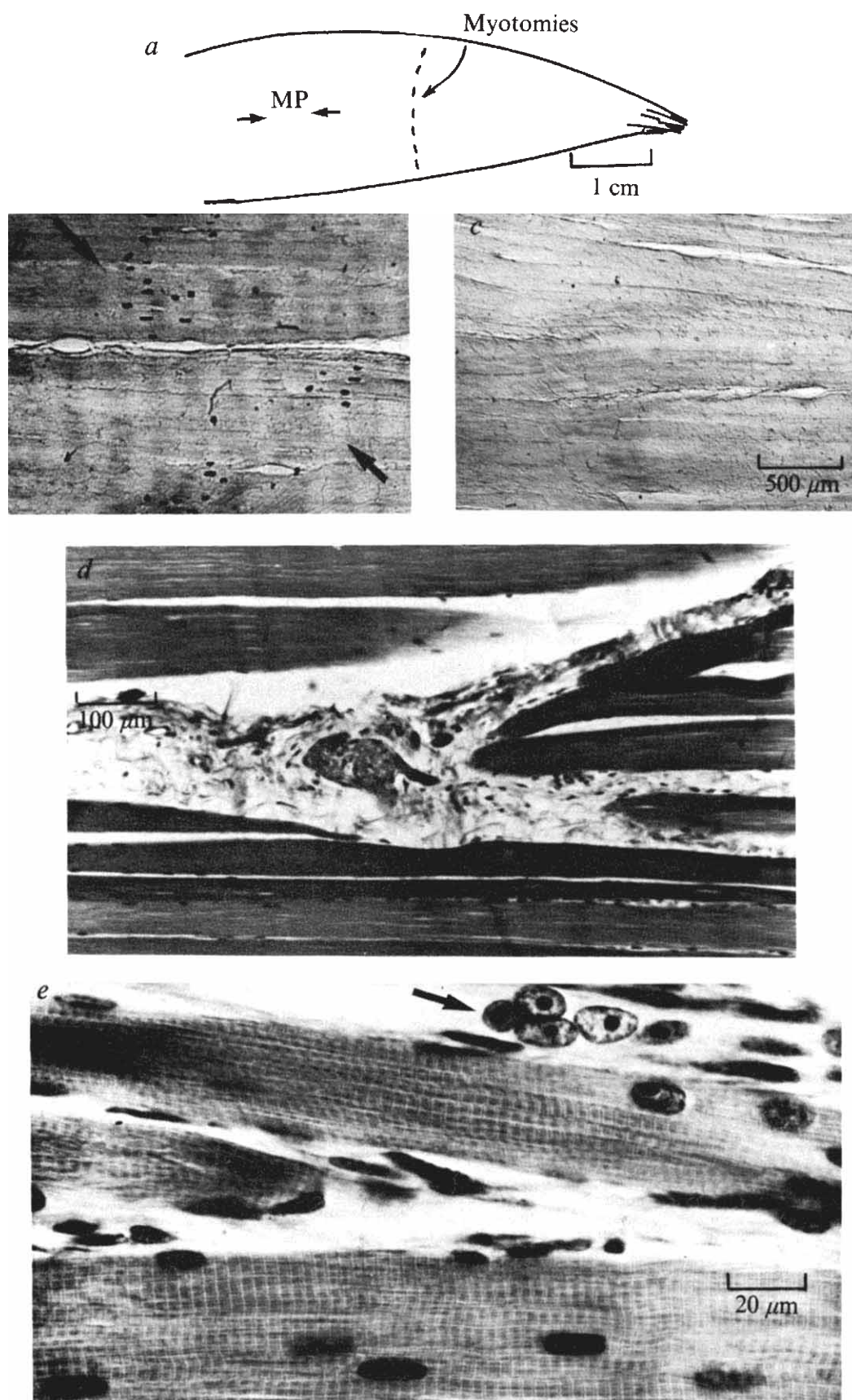


Fig. 1 Longitudinal histological sections in baboon biceps biopsied 2 months after extrajunctional myotomies. *a*, Sketch of the muscle with the motor point (MP) located by electrical stimulation and the distal myotomies. *b* and *c*, Acetylthiocholine method for staining motor endplate cholinesterase. *b*, Motor point region with many endplates (between arrows). *c*, Distal biceps near myotomies with no cholinesterase accumulations. *d* and *e*, Sections through myotomy area stained with Masson trichrome. *d*, Segmented muscle fibres (middle) next to intact muscle fibres (below). *e*, Myotube with four myoblasts (arrow).

were careful not to cause any lesion to the blood supply. The aponeurosis and skin were sutured. One to two months after the physiological observations a muscle biopsy was removed and longitudinal sections 5–10 μm were made. Staining for cholinesterase by the acetylthiocholine method disclosed motor endplates at the level of the previously identified motor point (Fig. 1*b*) whereas no focal cholinesterase accumulation was found throughout the distal part of the biopsy (Fig. 1*c*). Obviously the myotomies failed to involve any motor nerve fibre because they were quite distal to the innervation zone and intramuscular nerves. Some biopsy specimens were also stained with Masson trichrome, showing fairly normal isolated muscle fibre segments at the level of the myotomy (Fig. 1*d*). The segments had restored their membrane at the level of the lesion where myoblast proliferation and myotube formation were seen, with typical vesicular nuclei containing prominent nucleoli (Fig. 1*e*).

After the operation the animals were tested repeatedly after receiving a small intramuscular dose of tranquiliser (Sernylan or Thalamonal). The biceps muscles were explored with a sterile concentric needle electrode inserted through the skin, as for a human EMG. No fibrillation potentials were observed in the first 5 d. After 5 to 6 d, abundant fibrillations of typical triphasic waveform were recorded in many sites of the operated biceps, but only distally with respect to the myotomies (Fig. 2). No fibrillation was detected above that level where typical motor unit potentials could be elicited by stretching the muscle slightly. The presence of the needle electrode in the muscle did not trigger the fibrillation potentials because the latter could readily

be demonstrated with subcutaneous steel needles which did not enter the muscle itself (Fig. 2*a*). A few of the muscles were not tested before the tenth day to ensure that the fibrillations then recorded could not be ascribed to an inadvertent lesion possibly related to electrode insertions. The distal muscle fibre segments which fibrillated had obviously been deprived of neural influence since the time of the myotomy. On the other hand, when muscle fibres are denervated by neurotomy, the distal nerve stump takes about 2–3 d to degenerate in the baboon¹⁰ and it would be expected to maintain some trophic action on the muscle during this time¹¹. Two animals were prepared with biceps myotomy on one side and with a partial section of the biceps nerve on the other side. Spontaneous fibrillations started in the distal biceps with myotomy about 2 d earlier than in the partially denervated biceps. About 100 different fibrillation potentials were recorded after 10–20 d on either side in these animals. Most potentials were triphasic and their mean total duration was 3.1 ± 1.0 ms. The mean voltages were 136 ± 76 μV on the myotomy side and 112 ± 53 μV on the partially denervated side ($P < 0.01$). Whereas in deviated muscle fibres, fibrillations generally arise at the site of the old endplate¹² this is not an absolute rule¹ and our results indicate that fibrillations are generated in nerve-free segments of primate muscle (Fig. 2). Other changes characteristic of removal of neural trophic factors had been found in frog muscle segments—the appearance of acetylcholine receptors¹³ and the acceptance of motor innervation¹⁴.

The hypothesis that myopathic fibrillations result from muscle fibre segmentation is further supported by the finding

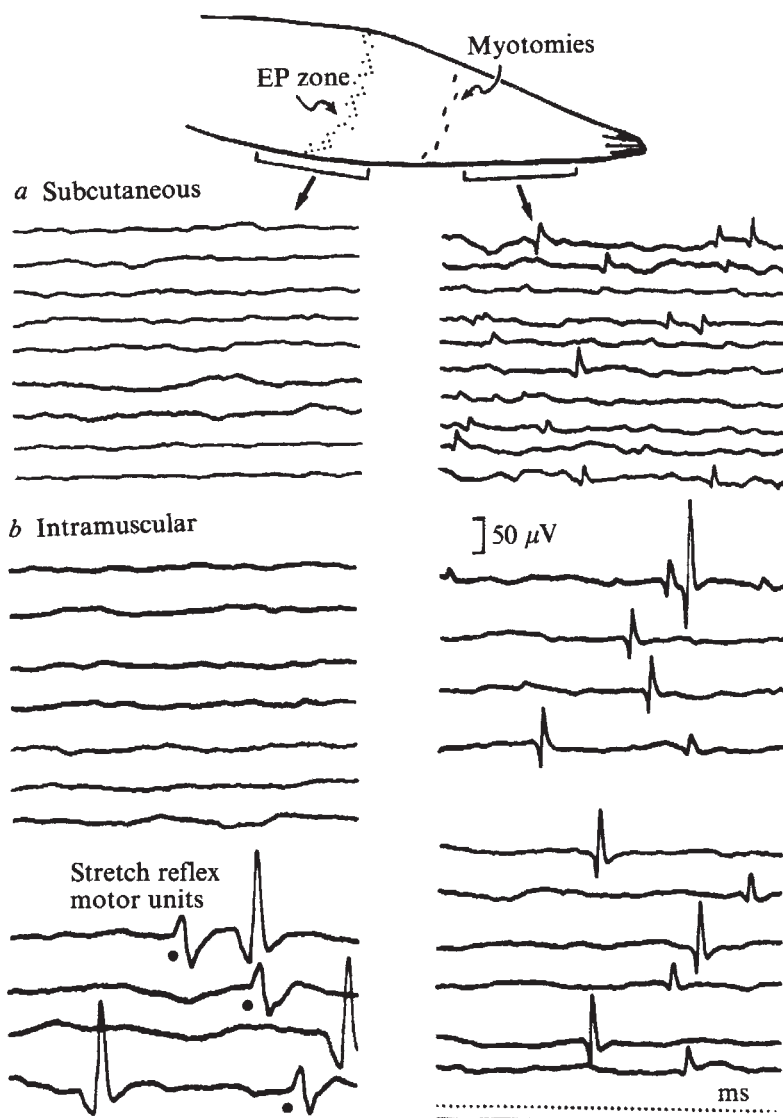


Fig. 2 Cathode ray oscillograms from baboon biceps muscle, 10 d after extrajunctional myotomy. Recording from the motor endplate zone (left column) and from the region distal to myotomy (right column). *a*, Subcutaneous bipolar needles not penetrating into the muscle. *b*, Concentric needle electrode in the muscle. Same calibration for all records. Fibrillation potentials are only recorded in distal region. At the bottom of the left column, a slight stretch elicited motor unit potentials and the one marked by dots starts with a negative (upwards) component which indicates focal recording at the endplate (see refs 8 and 9).

that spontaneous fibrillations are much more abundant than hitherto suspected in Duchenne muscular dystrophy^{4,15}, whereas they were very scarce, if present at all, in the muscles of five patients with typical facio-scapulo-humeral (Landouzy-Dejérine) muscular dystrophy. The most important pathological change in Duchenne muscular dystrophy is focal necrosis which isolates long fibre segments from the endplate region^{16,17} whereas this lesion is rare in facio-scapulo-humeral muscular dystrophy¹⁷. The correlation also holds for the muscles of patients with acute polymyositis which have marked lesions of focal necrosis¹⁸ as well as abundant fibrillations in EMG tests. Because they generally looked at transverse muscle sections most pathologists failed to emphasise that myopathic necrotic lesions, far from destroying the muscle fibres, result in serially arranged fibre segments. That nerve-free segments thus formed are collaterally innervated and can then be activated by the motor nerve in Duchenne muscular dystrophy^{7,15}, and also in polymyositis¹⁹, sheds new light on the pathogenesis and emphasises regenerative capabilities which must retard the clinical progression and/or promote the recovery of human myopathies with muscle fibre segmentation.

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Electrical behaviour of inner and outer smooth muscle of sheep carotid artery

MICROELECTRODE studies of inner smooth muscle cells of unstimulated rabbit carotid arteries show a stable membrane potential^{1,2}, as do sucrose-gap records from sheep carotid arteries^{3,4}, the latter method records only from cells that are widely interconnected electrically. Information about the electrical behaviour of the outer muscle of arteries is particularly important as only the outer muscle is innervated, and it is much less sensitive than inner muscle to vasoconstrictor agents⁵.

We have now been able to record from the outer, as well as the inner, muscle of the sheep carotid artery by microelectrodes after stripping away successive layers of tissue from the inner side of the artery. Electrotonic potential was applied through external electrodes⁶. The strips of artery used were approximately 3 mm wide and 15 mm long, their long axis being aligned round the artery wall and parallel to the long axis of their smooth muscle cells. The bathing solution contained (mM): Na⁺ 137.4; K⁺ 5.9; Mg²⁺

1.2; Ca²⁺ 2.5; Cl⁻ 134.0; HCO₃⁻ 15.5; H₂PO₄⁻ 1.2 and glucose 11.5. It was gassed by 95% O₂: 5% CO₂ and maintained at 36–37 °C.

Microelectrodes inserted into inner cells through the intact intimal surface of the vessel recorded a stable membrane potential of -59.5 ± 5.3 mV (mean $\pm$ s.d., 33 experiments). Spontaneous electrical activity was never observed in these cells. Electrotonic hyperpolarising currents caused hyperpolarisation proportionate to the current intensity.

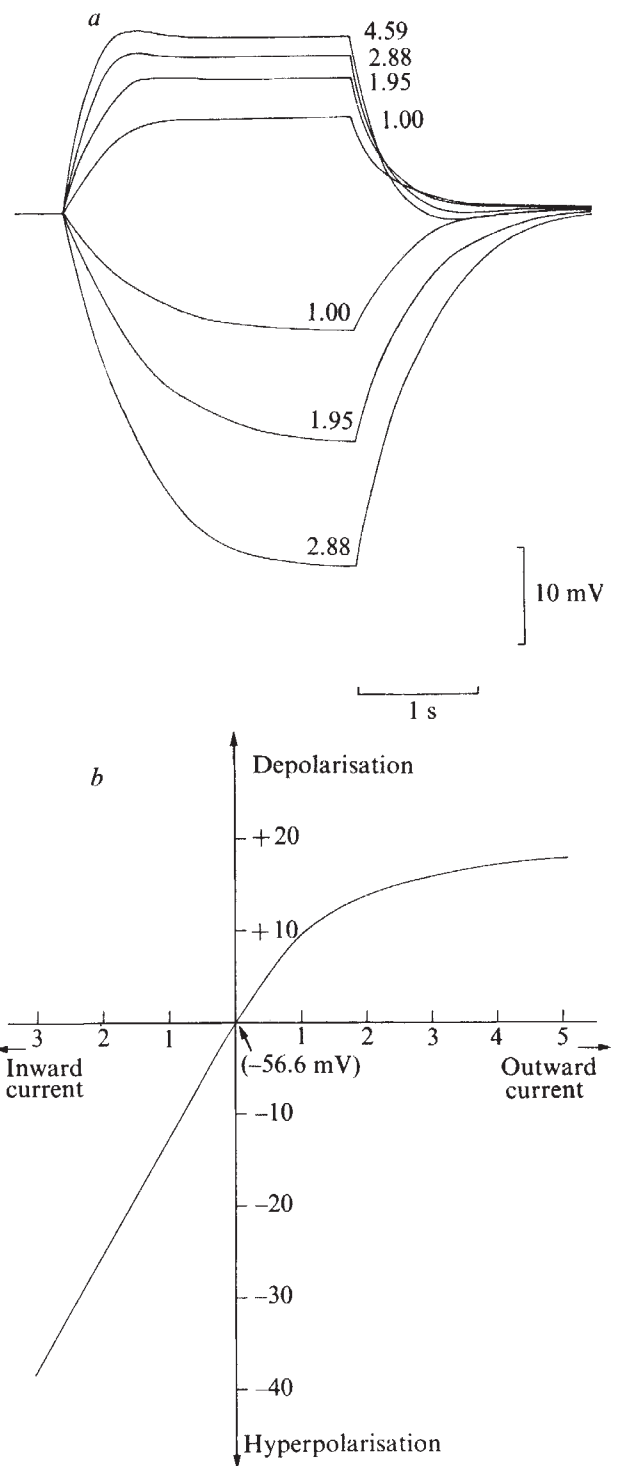


Fig. 1 *a*, Electrotonic potential evoked by external current application to the inner muscle of the sheep carotid artery. *b*, Voltage-current relationship for steady state readings. Values for current in arbitrary units.

Depolarising currents caused proportionately less depolarisation when the membrane potential passed approximately -40 mV and the final level of depolarisation was then often less than was initially produced (Fig. 1). This delayed rectification on depolarisation is very similar to that seen in inner cells of rabbit aorta⁷ in which it was attributed to an increase in potassium permeability. Depolarising currents never produced a series of electrical discharges. Similar results were obtained from inner muscle cells after stripping off the intima and a layer of adjacent muscle about $50\text{ }\mu\text{m}$ thick; membrane potential was stable at 60.0 ± 5.5 mV (mean $\pm$ s.d., 30 experiments) and electrotonic current produced changes similar to those in Fig. 1. The space constant of inner muscle measured by electrodes inserted at increasing distances from the polarising electrodes was

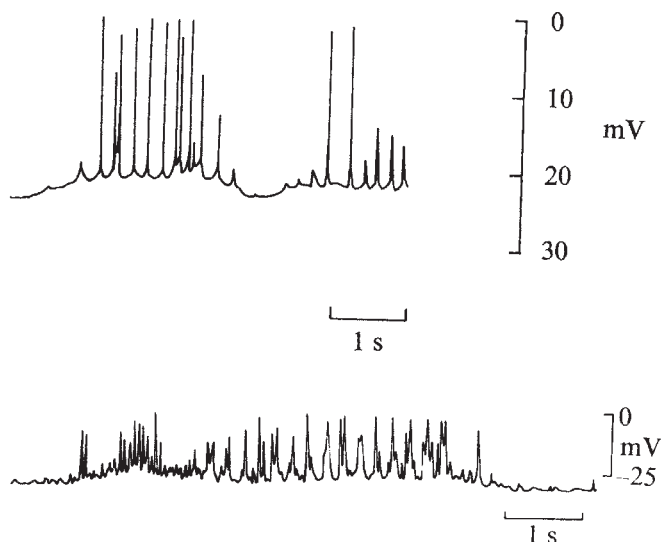
Table 1 Membrane constants of smooth muscle of the sheep carotid artery

Intact inner muscle		Stripped inner muscle	
Experiment no.	Space constant (mm)	Experiment no.	Space constant (mm)
1	1.75	2	2.04
3	1.77	6	1.98
4	1.87	8	2.14
5	2.00	13	2.18
7	1.99		
9	1.73		
10	2.27		
11	1.74		
12	2.43		
Means	1.95 (s.d. $\pm$ 0.24)		2.09 (s.d. $\pm$ 0.08)

always between 1.74 and 2.43 mm (Table 1) which is similar to the value of 1.26–3.49 mm obtained for these arteries by the sucrose-gap method⁸.

When further layers were stripped away from the inner part of the artery wall and microelectrodes inserted from the stripped surface into the outermost muscle, irregular spike discharges were recorded in every case (Fig. 2), which were sometimes superimposed on slow oscillations. The membrane potential between spikes was only -31.1 ± 4.4 mV and spike height was 28.8 ± 5.7 mV (means $\pm$ s.d., 30 experiments). Overshoot of the spike beyond zero potential was rare. No electrotonic potential could be induced in the outer cells even with the nearest polarising electrode only 0.25 mm from the recording site. Attempts to record from

Fig. 2 Action potentials recorded from the outer muscle of sheep carotid artery.



outer muscle by inserting microelectrodes from the outer surface, after stripping the adventitia, were not successful.

The low resting potential and spontaneous activity of outer muscle might have resulted in part from the stripping of adjacent tissue that was necessary to expose it; however, as a similar procedure failed to induce any activity in inner muscle, the outermost cells of the artery clearly developed electrical activity more readily than inner cells. They also differed in lacking widespread electrical contacts with neighbouring cells. These properties would allow electrical discharges in outer muscle to have an important, and localised, function in mediating its response to nerve. The relative electrical stability of inner muscle, attributable to powerful delayed rectification, and long space constant, make it adapted for extensive conduction of steady depolarisation such as that induced by injury⁸.

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Possible biochemical explanation for specific reformation of synapses between muscle and regenerating motoneurons in cockroach

THE reformation of synaptic connections by a neurone whose axon has been severed often proceeds with great specificity so that synapses are reformed only with the original cell. It is possible that this occurs as a result of a specific recognition process at the surface of the cells. Evidence for such a process can be obtained by biochemical analysis. The neuromuscular system of the cockroach *Periplaneta americana* leg is a system which demonstrates specificity of synapse formation by regenerating motoneurons¹ and which lends itself to such an investigation.

The set of coxal depressor muscles (CDMs) in each leg are composed of individual muscles each of which can be identified and numbered². Six of the CDMs in the metathoracic legs, 178, 179, 177d', 177d, 177e' and 177e are particularly suitable for such studies because their pattern of innervation by identified motoneurons in the metathoracic ganglion has been determined³. All the fibres in muscles 178 and 179 are innervated by the same identified, fast motoneurone, cell 28 in the map by Cohen and Jacklet⁴. In muscles 177d and 177e all the fibres are innervated by the same slow motoneurone on the dorsal surface of the ganglion, probably cell 52, and in addition by three inhibitory motoneurons. Muscles 177d' and 177e' possess the unusual characteristic of having every fibre innervated by both motoneurone 28 and 52 (ref. 3) so that they are an homogeneous population of doubly innervated fibres with no inhibitory innervation. Another advantage to using this cockroach system is that it has been demonstrated that after the nerves to the CDMs have been cut and regeneration of the neurones has proceeded so that movement is recovered, the excitatory motoneurons have specifically reinnervated the original muscles⁵. This is the only system where specificity of synapse formation during regeneration has been demonstrated at the level of

individual, identified cells. The CDMs are large enough for some biochemical analyses to be done on tissue from a single cockroach. The amount of protein in the various CDMs from the adult male cockroach is sufficient for polyacrylamide gel electrophoresis of muscle proteins from a single animal but not sufficient for biochemical studies requiring bulk amounts of material (178, 179, 1.3 mg; 177d, 80 μ g; 177d', 70 μ g; 177e, 60 μ g; 177e', 90 μ g).

If the regenerating motoneurons make synapses with the correct muscle as a result of a specific recognition involving macromolecules on the surface of the muscle then one should be able to distinguish biochemically the various CDMs. This was surprisingly easy to do. When unfractionated homogenates of each of the six CDMs are analysed by sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis⁶, three different gel protein patterns are observed (Fig. 1). All the muscles possessing one particular protein pattern also have the same innervation. In the doubly innervated muscles, 177d' and 177e', the gel protein pattern seems to be a composite of those protein bands correlated with each of the two individual motoneurons. Innervation of a muscle by motoneurone 28 is accompanied by the bands labelled F1, F2, whereas innervation by motoneurone 52 is accompanied by bands S1, S2, S3, S4, S5, S6, S7. Innervation by the inhibitory motoneurons is accompanied by band S8. More than fifty individual cockroaches have been examined in the course of this study and the association of a particular gel protein pattern and innervation type has been observed in every one.

If any of these protein differences are contributing to the specific recognition of the CDMs by the regenerating motoneurons it must be demonstrated that they are present in the muscle throughout the period of neural regeneration and that their synthesis by the muscle is not controlled by the motoneurone. The nerves were therefore cut and the denervated CDMs analysed at various times after the operation by SDS-polyacrylamide gel electrophoresis as in Fig. 1. No change is observed in the gel protein pattern of the six CDMs as a result of denervation up to 8 weeks after the operation. The nerves were recut every 3 weeks to make sure that the motoneurons did not reconnect with the muscles. Eight weeks after denervation, the muscles have atrophied to such an extent (less than 40% normal protein content) and become so covered with fat cells and haematocytes that a clean dissection of muscle tissue is impossible.

This experiment therefore demonstrates that throughout the period of regeneration of motoneurons (30–60 d for initial contact) the six CDMs can always be distinguished by their gel protein patterns.

The failure of denervation to change the gel protein pattern of the CDMs may be because the proteins have a long half life or are continually synthesised. The nature of newly synthesised proteins from the CDMs was examined by measuring the distribution of radioactivity in the SDS electrophoresis gel after running a sample containing ³H-leucine incorporated into muscle proteins. It is observed (Fig. 2a) that the pattern of radioactivity in the innervated, control CDMs is not identical with that pattern of stained bands. Discrepancies may be accounted for by different turnover rates for the various proteins. Although it was very easy to distinguish the different CDMs by their gel protein pattern, it is more difficult, but still possible, to do so by examining the patterns of radioactivity. When the radioactive proteins of molecular weight less than 35,000 are examined on an expanded scale however, the differences become more apparent (Fig. 2b). The different amounts of F1, S1, S2, S3 synthesised by the various CDMs are clearly seen. Although the patterns of newly synthesised proteins are changed when the CDMs are denervated (Fig. 2c), they can still be clearly distinguished from each other as seen in Fig. 2d.

Mammalian skeletal muscle responds quite differently from cockroach muscle to denervation. On denervation of mammalian slow or fast skeletal muscles physiological and biochemical parameters change to values intermediate between those of normal fast and slow fibres⁷. An apparent dedifferentiation of both types of fibres to a common, simplified type has occurred. This loss of relative biochemical identity may account for the lack of specificity seen in its interactions with regenerating mammalian motoneurons which seem to be able to reinnervate any denervated muscle⁸. It is from these differences in response to denervation that we seek to explain the specificity observed in the cockroach neuromuscular system. The observed ability of the various CDMs to maintain their biochemical identity after denervation and throughout the entire period of regeneration of motoneurons must be an absolute requisite for specific reinnervation to occur by a process requiring a specific recognition between the cells.

Although the various CDMs have different mechanical

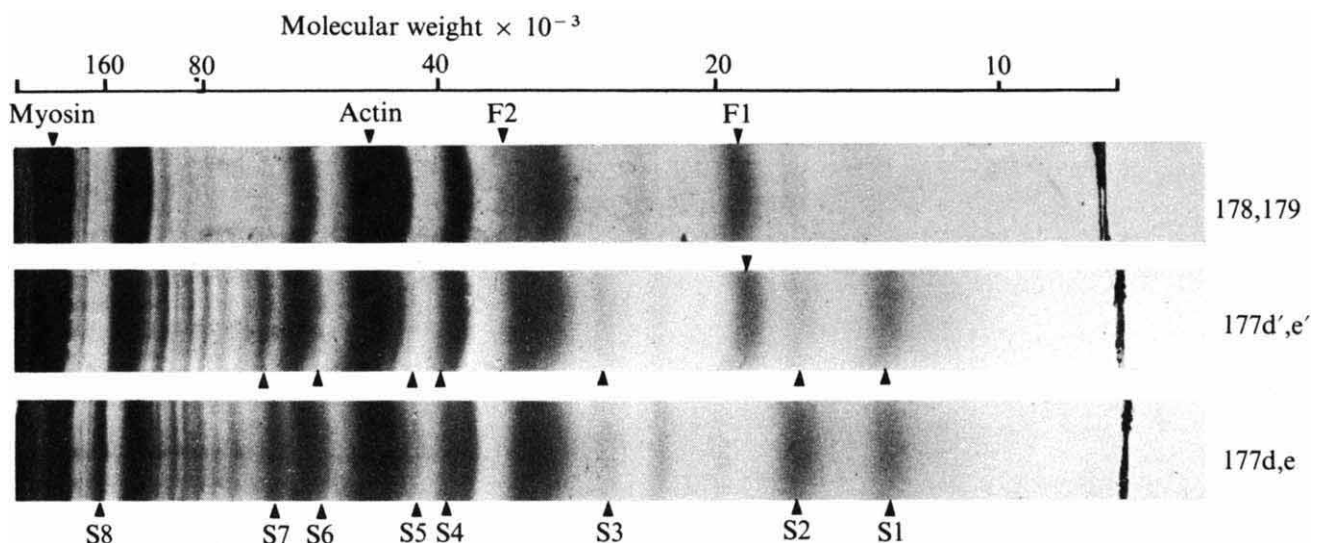


Fig. 1 SDS-polyacrylamide gels of the coxal depressor muscles stained with Coomassie blue. Each of the six CDMs was run individually but only the three different characteristic patterns are shown. The isolated muscles were homogenised in the appropriate volume of 0.01M Tris-HCl (pH 7.4), 10% sucrose, 1% SDS, and 5% 2-mercaptoethanol and heated in a boiling water bath for 5 min. Aliquots of these solutions were applied to 10% polyacrylamide gels which were run in the presence of 0.2% SDS, stained with Coomassie blue, and destained all according to the methods of Fairbanks *et al.*⁶.

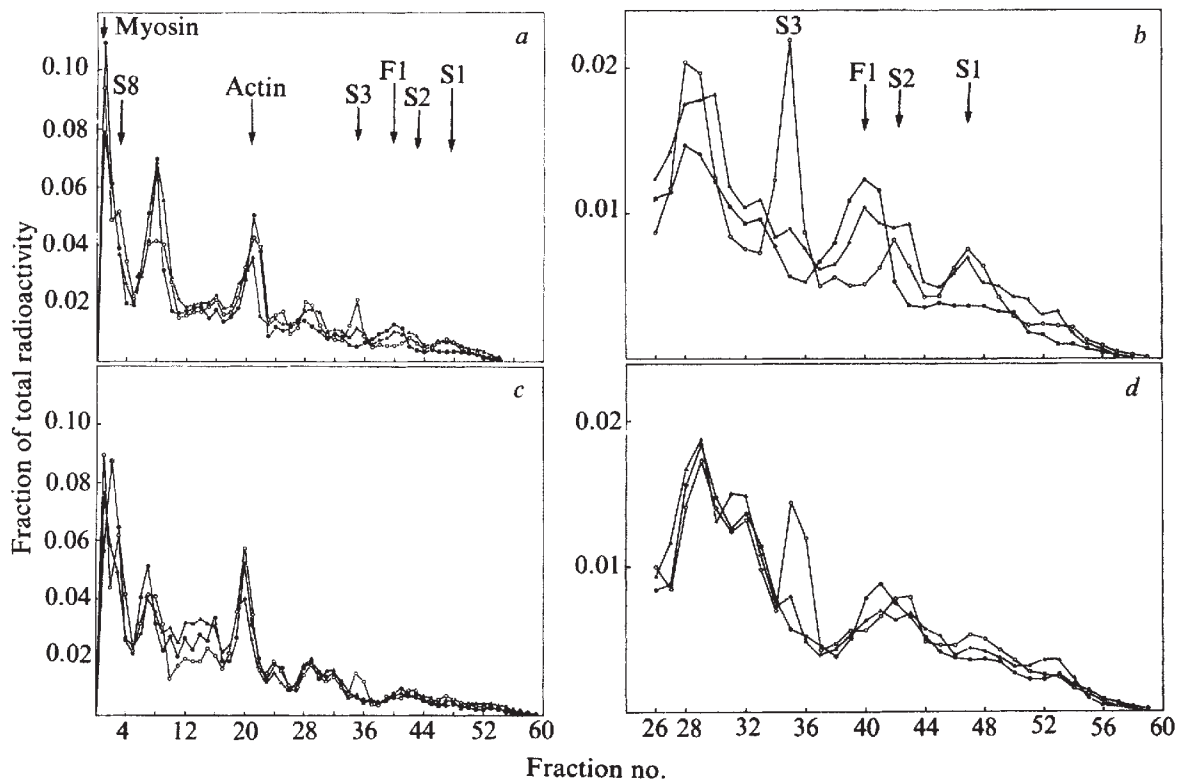


Fig. 2 Distribution of radioactivity after SDS-polyacrylamide gel electrophoresis of CDM homogenates after ^3H -leucine incorporation *in vivo*. Innervated control CDM (a) and (b) and CDM denervated 4 weeks before experiment (c) and (d) are taken from different metathoracic legs of the same animal. (b) and (d) are portions of (a) and (c) respectively but with expanded axes. The various CDMs are 178, 179 (●); 177d, 177e (○); 177d', 177e' (△). ^3H -leucine (specific activity 46 Ci mmol $^{-1}$) was concentrated twentyfold and 5 μl containing 117 μCi were injected into the coxa of each metathoracic leg just above the CDMs. After 22 h the muscles were removed, homogenised in sample solution, prepared for electrophoresis, run on gels, run through staining procedure (without Coomassie blue), and destained as described in Fig. 1. The gels were sliced into 1-mm sections and allowed to dry overnight. One millilitre of NCS (10% water) tissue solubiliser was added to each slice in a vial and they were incubated at 45 °C for 48 h. Toluene scintillation fluid was added and samples counted. Double-labelling experiments were not done because it was felt to be more important to have control, innervated and experimental, denervated muscles come from the same animal. The techniques were sufficiently precise that qualitatively similar results were obtained in each of three experiments.

properties it has been demonstrated that these are produced by differences in neuromuscular transmission and not by different contractile mechanisms in the muscle⁹. Therefore, the protein differences observed are not likely to be involved with contractile processes. It has not been demonstrated that any of the protein differences have a functional role in the specific recognition of the muscle by the regenerating motoneurone. It seems highly likely, however, that if such proteins exist they would be expected to express the following properties. (1) Their presence in a muscle should be associated with innervation by a particular motoneurone. (2) They should be present in the muscle throughout the period of regeneration of the motoneurons. (3) Their synthesis should be an endogenous property of the muscle and not under trophic control of the motoneurone. The protein bands described in this communication that serve to characterise a particular CDM possess these properties.

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Skin collagen allografts in the rat

THE factors involved in skin graft rejection have not been elucidated fully but it is known that the cellular components of skin, including the epidermis and its appendages, vascular endothelium and passenger leukocytes, are of primary importance in the process. Such cellular elements can be removed by treating skin with a solution of crystalline trypsin to leave the dermal collagen component intact. A preliminary study of allografts of trypsin purified dermal collagen (hereafter referred to as dermal collagen) in the pig showed that although subcutaneous implants underwent some initial collagenolysis they achieved a state of permanence without lymphocytic infiltration occurring³. Since this concurred with the view that mature collagen shows low or no antigenicity, especially within species^{4–7}, we thought it likely that trypsin prepared collagen could be transplanted in animals other than the pig without eliciting an immune response.

Here we report on the fate of dermal collagen allografts, using the same outbred strains or differing strains of rat as donors and recipients, in an evaluation of dermal collagen

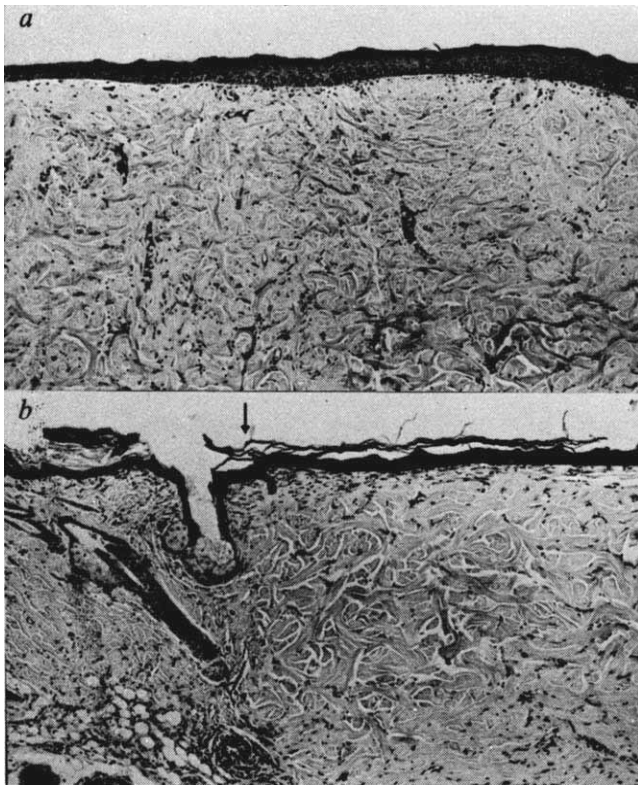


Fig. 1 Trypsin prepared ASH/W dermal collagen, treated with 0.01% glutaraldehyde then grafted into full thickness loss excised skin wounds in Porton recipients (haematoxylin and eosin, $\times 135$). *a*, 28 d after grafting. The recipient epidermis which has migrated over the graft is hyperplastic and has a parakeratotic surface layer. *b*, 27 d after grafting. Recipient skin is at left of graft junction (arrow). The epidermis over the graft is of nearly normal thickness and has a stratum corneum. Dermal collagen was prepared by treating full thickness skin with a solution of crystalline trypsin (Koch-Light) at a concentration of 2 mg ml^{-1} in 0.1 M phosphate buffer solution, pH 9.0, with 0.5 mg ml^{-1} sodium azide as a bactericide at 15°C for 28 d. The collagen was then treated with 0.01% glutaraldehyde (Taab Laboratories) in Tris buffer, pH 7.2 for 16 h at 15°C before washing and grafting. Glutaraldehyde is known to desensitise collagen to the activity of collagenase².

for the replacement of collagenous tissues and, in particular, skin.

To confirm skin antigenicity, 31 skin allografts and 15 autograft controls were grafted into subcutaneous sites or excised skin wounds, biopsied 5–84 d after operation and examined histologically. In contrast with the autografts, the allografts developed the characteristic features of graft rejection, that is, rapid infiltration with lymphocytes, destruction of the epidermis and its appendages within 7–14 d and variable lysis of the graft collagen.

A total of 84 dermal collagen allografts were then implanted subcutaneously and biopsied 3–83 d after operation. These included 44 implants pretreated with solutions of glutaraldehyde at concentrations ranging from 0.001 to 1.0%. The implants, which were all recovered intact, became recellularised and revascularised with no overt evidence of lymphocytic infiltration or of collagenolysis. The original collagen bundle architecture was especially well preserved in implants treated with 0.01% glutaraldehyde, but persistent inflammation was associated with those treated with higher concentrations. The antigenic status of dermal collagen was investigated further by sensitising six rats to allogenic skin by making subcutaneous implants 14 or 28 d before implantation of collagen from the same donor. The collagen implants, which were recovered 14–48 d after operation, were no different from those implanted in

non-sensitised rats and in particular showed no signs of lymphocytic infiltration.

Having established that allograft dermal collagen was neither resorbed nor induced an immune response in subcutaneous sites, we then investigated the possibility of incorporating dermal collagen into cutaneous wounds. Thirty-two collagen allografts (including 10 pretreated with 0.01% glutaraldehyde) were grafted into full thickness loss excised wounds which were examined, measured and biopsied 4–59 d after operation.

It was important to minimise graft desiccation and a foam dressing as used on five of the grafts proved ineffective in this respect. Ten of the wounds (seven measuring $5 \times 5 \text{ mm}$ and three measuring $10 \times 10 \text{ mm}$) were simply covered with an overlapping sheet of dermal collagen then dressed with Vaseline petroleum jelly gauze. Here the outer aspect of the collagen became desiccated but the underlying collagen not only became recellularised and revascularised but also progressively epidermalised with recipient epidermis (Fig. 1*a*).

A further 17 $10 \times 10 \text{ mm}$ wounds received fitting collagen grafts and were dressed with a sheet of dermal collagen then Vaseline gauze. The collagen dressings desiccated and since the grafts became epidermalised by migration either at the graft–dressing boundary or within the superficial aspect of the grafts the whole or greater thickness of the grafts became incorporated into the wounds (Fig. 1*b*). Epidermalisation resembled that described for healing incisional wounds^{8,9} except that it occurred over a large area and took 4–5 weeks for completion in the $10 \times 10 \text{ mm}$ grafts. The wound epidermis was initially hyperplastic with an outer parakeratotic layer (Fig. 1*a*) but, eventually assumed a normal thickness with a stratum corneum (Fig. 1*b*).

The grafts as measured 3–8.5 weeks after operation, ranged from 60 to 100% of their original area, with best size maintenance in the 0.01% glutaraldehyde treated grafts. The efficacy of the grafts in suppressing wound contraction can be demonstrated by comparing them with excised wounds and full thickness autografts of the same initial size. Excised wounds without dressings contracted to hairline scars by 14 d and by 32 d when covered with a Vaseline gauze dressing. Autografts maintained their size for 2–3 weeks then diminished to 50–60% of their original area by 5 weeks (figures of 45–55% have been reported in a comparable study¹⁰).

The desiccated collagen dressings (or superficial aspect of the grafts) formed a hard outer crust which seemed to prevent fluid loss and bacterial penetration. None of the grafts became infected and it may be pertinent that decreased bacterial counts are found in experimentally infected burn wounds in rats dressed with a bovine skin collagen preparation¹¹.

Thus, it might prove possible to use dermal collagen allografts for the permanent replacement of lost or damaged skin in humans as well as animals. The long term fate of such grafts is currently under investigation.

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Melanoma cells which require cyclic AMP for growth

THE cyclic forms of AMP, GMP and CMP have been implicated as agents which either stimulate or inhibit cell division. Pastan *et al.* reviewed this topic¹ and others have dealt with it²⁻⁶. Our studies of the growth of cultured melanoma cells have focused on the inhibitory effects of cyclic AMP⁷. We analysed variant cells that can grow in the presence of agents, such as melanocyte stimulating hormone (MSH), which increase the intracellular levels of cyclic AMP and inhibit the growth of wild-type cells. We describe here a class of variants which are not only resistant to the inhibition, but require cyclic AMP to grow at the rate normally observed in wild-type cells. In one such variant, division was markedly stimulated when either MSH, theophylline, isobutyl methyl xanthine, cholera toxin, prostaglandin E₁ (PGE₁) or dibutyryl cyclic AMP was added to the medium. Each agent inhibited the growth of wild-type cells. The cells were temperature-sensitive in that they expressed the variant phenotype at 37 °C, but behaved as the wild type at 40 °C. Studies of dose responses to dibutyryl cyclic AMP revealed that growth was also enhanced in the wild type by lower concentrations of the nucleotide (10⁻⁵ M), a fact that had gone unnoticed in melanoma cells but has been reported for other cell types¹. These findings suggest a stimulatory as well as inhibitory role for cyclic AMP in control of growth of melanoma cells.

The wild-type cell line was a pigmented subclone of the Cloudman S91 melanoma which was selected for loss of hypoxanthine-guanine phosphoribosyl transferase activity⁸. This line was designated PSI-HGPRT-1. We exposed these cells to the mutagen N-methyl-N-nitro-N-nitrosoguanidine by standard procedures⁹ and then selected for clones that were resistant to the inhibition of growth which occurs when MSH and theophylline are included in the culture medium. One clone, described below, required cyclic AMP for normal rate of growth and was designated PSI-HGPRT 1-cA^{dep}. This clone will be referred to here as cA^{dep}.

Cells of cA^{dep} grew poorly in normal F10 culture medium but had division rates similar to wild-type cells when the medium was supplemented with agents known to increase the intracellular levels of cyclic AMP (Fig 1a). In contrast, the growth of wild-type cells was inhibited by these agents (Fig. 1b). For example, in a typical experiment, the mean doubling time of cA^{dep} cells was 96 h in normal F10 medium and 48 h in medium supplemented with MSH (2 × 10⁻⁷ M) and theophylline (2 × 10⁻³ M). For wild-type cells the doubling time was 41 h in normal F10 and 89 h in the supplemented medium. MSH or theophylline alone, dibutyryl cyclic AMP (2 × 10⁻⁴ M), PGE₁ (4 × 10⁻⁵ M), isobutyl methyl xanthine (1 × 10⁻⁴ M, data not shown) and cholera toxin (0.5 µg ml⁻¹, data not shown) each markedly stimulated the growth of cA^{dep} cells and inhibited the growth of wild-type cells. The cA^{dep} cells were temperature sensitive for the expression of the variant phenotype. They behaved as wild-type cells when cultured at 40 °C (Table 1).

Studies of dose-response to dibutyryl cyclic AMP showed that division by wild-type cells was maximally stimulated by a concentration of about 10⁻⁵ M. This was ten times

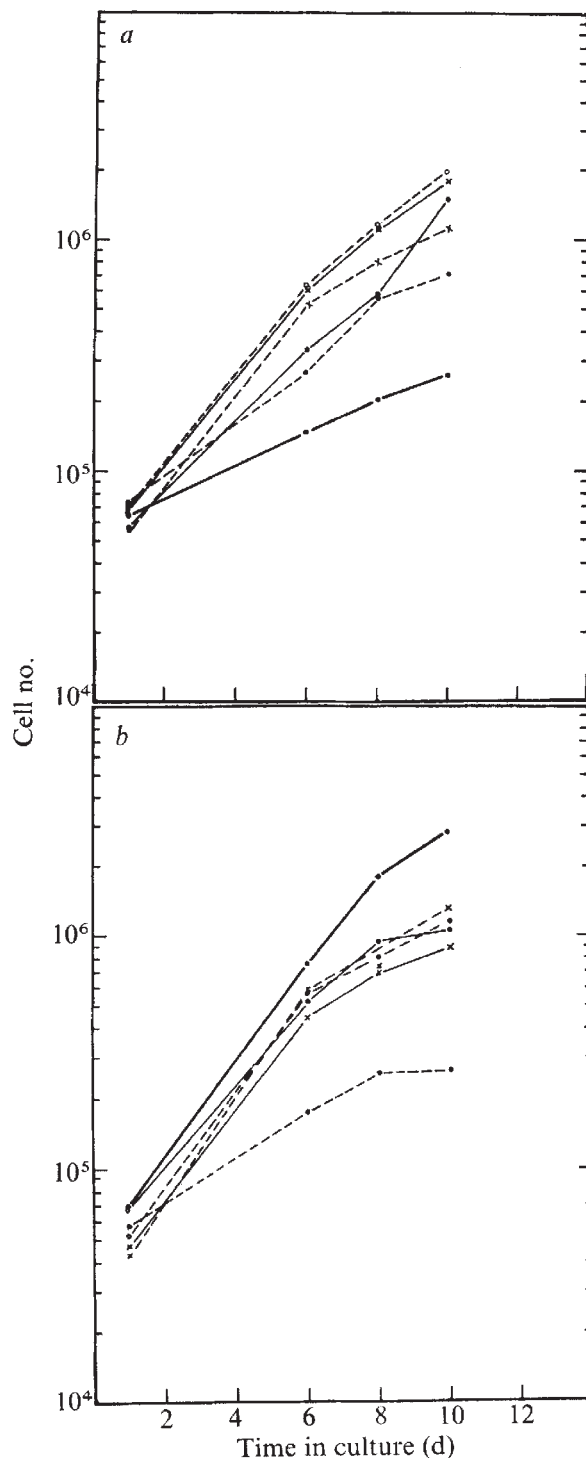


Fig. 1 Growth of cA^{dep} (a) and wild-type cells (b) with various additives in the culture medium (see text). Cells (5 × 10⁴) were inoculated into 4 ml F10 culture medium supplemented with 2% foetal calf serum and 10% horse serum in 30-ml Falcon tissue culture flasks. The temperature was maintained at 37 °C. Growth curves were constructed by counting cells from triplicate culture flasks in a Coulter counter. Medium was changed at 3-d intervals. Prostaglandin E₁ was given by Dr John E. Pike (Upjohn). ●—●, Control; ×—×, PGE₁; ○—○, dibutyryl cyclic AMP; □—□, theophylline; ×—×, MSH; ●—●, MSH and theophylline.

Table 1 Doubling times of cA^{dep} cells at 37 and 40 °C

	Length of cell cycle (h)	
	37 °C	40 °C
Normal medium	62	38
Medium + MSH	43	64

Cells (5×10^4) were cultured for 7 d in normal medium or medium containing MSH (2×10^{-7} M). Cultures in triplicate were then collected and counted in a Coulter counter. These experiments were repeated three times with similar results each time. Growth of wild-type cells was inhibited by MSH at both temperatures (data not shown).

lower than that for optimal stimulation of cA^{dep} cells (Table 2). As would be expected, we encountered some variability in the dose-response studies but the tenfold difference in optimal concentration remained constant. That the cells lacked HGPRT was not related to this phenomenon since wild-type cells that had normal levels of HGPRT were also stimulated by dibutyryl cyclic AMP (data not shown). It should also be noted that cA^{dep} and wild-type cells were always inhibited in growth by concentrations of dibutyryl cyclic AMP of 8×10^{-4} M or higher.

There has been some question about the specificity of the stimulation of growth by low concentrations of dibutyryl cyclic AMP since other compounds such as adenosine can elicit the same response (see for example, refs 1 and 10). We saw the stimulation, however, only with the cyclic forms of AMP. In an experiment parallel to that in Table 2, 5'-AMP, 3', 5'-cyclic GMP and its dibutyryl derivative were added to wild-type and cA^{dep} cells in concentrations ranging from 1×10^{-6} M to 5×10^{-4} M, and none of these compounds affected the growth of the cells. We conclude that the stimulation of growth of wild-type cells by the lower concentrations of dibutyryl cyclic AMP is a specific effect of the cyclic nucleotide.

Table 2 Doubling times of wild-type and variant cells at various concentrations of dibutyryl cyclic AMP

Molarity of dibutyryl cyclic AMP in culture medium	Length of cell cycle (h)	
	Wild-type cells	cA ^{dep} cells
0	56	80
1×10^{-6}	34	58
4×10^{-6}	35	50
1×10^{-4}	39	42
2×10^{-4}	42	43
4×10^{-4}	53	53
8×10^{-4}	80	120

Cells (5×10^4) were inoculated into 4 ml F10 culture medium supplemented with 2% foetal calf serum and 10% horse serum in 30 ml Falcon tissue culture flasks. After 24 h the medium was changed and dibutyryl cyclic AMP was added at the concentration indicated. Growth curves were constructed by counting cells from triplicate culture flasks in a Coulter counter during 11 d. Medium was changed at 3-d intervals.

Reports from many laboratories have shown that cyclic AMP can inhibit the proliferation of various cell types. Far fewer reports have indicated that cyclic AMP has a mitogenic action as well (for example, refs 1 and 4). For example, it has recently been shown that DNA synthesis commences in normal rat livers when the animals are injected with dibutyryl cyclic AMP and theophylline². Also, cyclic AMP levels increase after partial hepatectomy and remain high during the prereplicative period, suggesting that increased cyclic AMP levels signal the onset of liver regeneration³. Cyclic AMP may also trigger cell division in lymphocytes⁶. The temperature-sensitive cA^{dep} variant de-

scribed here strengthens the possibility that cyclic AMP produces both kinds of effect physiologically in melanoma cells. Analyses of this variant should be useful for dissecting the mechanisms, and we are investigating the molecular intermediates involved. It will be interesting to determine if cyclic AMP-dependent variants exist among other cell types. Such variants could provide evidence for a generalised dual role of cyclic AMP in the control of cell proliferation.

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Molecular structure of nicotinamide adenine dinucleotide

NICOTINAMIDE adenine dinucleotide (NAD) has a fundamental role in metabolic processes as an electron transport molecule. Although its chemical structure was elucidated¹ in 1934, its detailed conformation remains still to be established in spite of numerous physicochemical applications². NAD analogues with a variety of substitutions on the bases are known to retain considerable activity of the natural coenzyme as long as the pyrophosphate diester group has been retained^{3,4}. The geometry of this backbone moiety is therefore indispensable to our understanding of the conformation and function of the coenzyme. We have so far no experimental evidence on this in NAD or any other nucleotide coenzyme molecule. X-ray studies have been possible only on those analogues^{5,6} where the nicotinamide and adenine rings are linked by a trimethylene bridge. The results are conflicting and it is difficult to use them to provide a structural basis for the NAD molecule itself, particularly as the phosphate backbone is absent from these analogues.

We have determined⁷ the molecular structures of cytidine-5'-diphospho-choline (CDP-choline) and CDP-5'. The study provides for the first time the detailed geometry of the pyrophosphate diester linkage present in a nucleotide coenzyme as well as its orientation with respect to the ribose moiety. The study shows further that the observed geometry is fully compatible with ester linkages to nucleo-

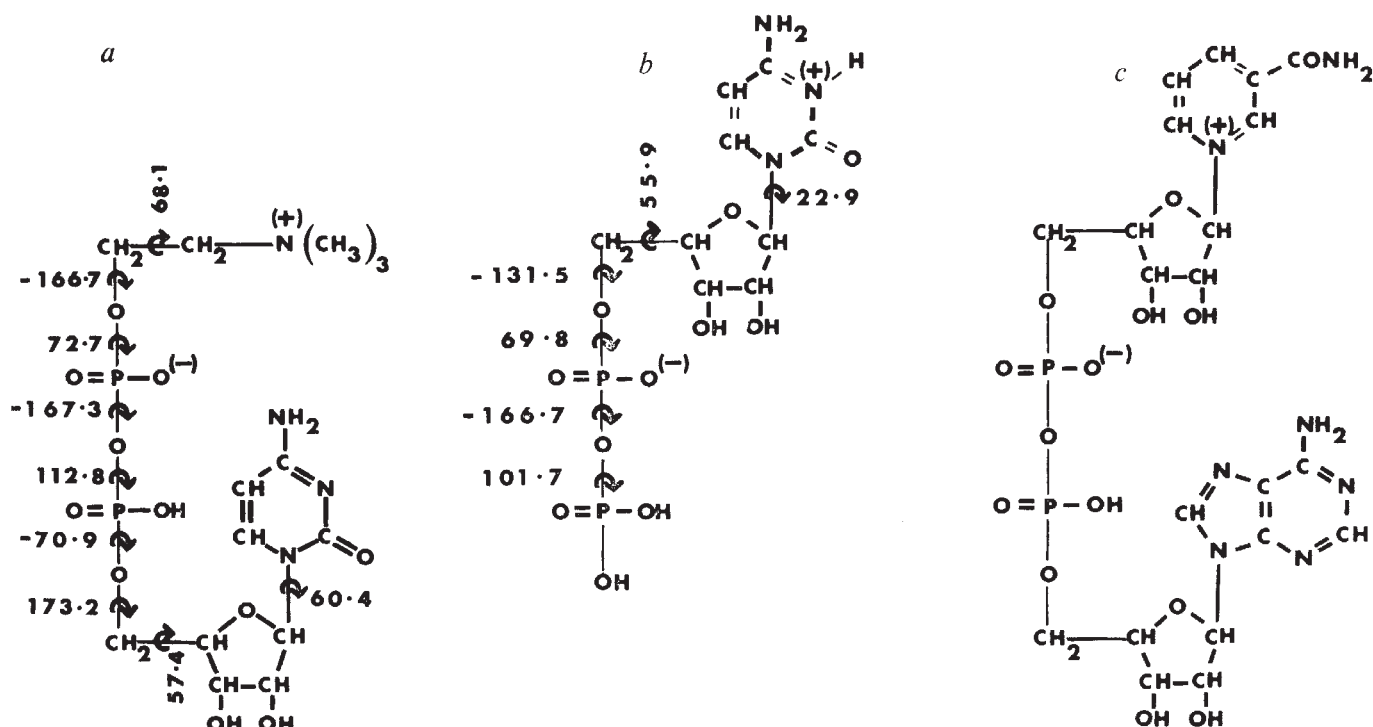


Fig. 1 Chemical structures of CDP-choline (a), CDP (b) and NAD (c). Torsional angles (conventions as in reference 11) observed in the crystal structures are also shown. The geometry and orientation of the pyrophosphate group in CDP are those observed in CDP-choline from the choline end, that is, as if the choline moiety is replaced by cytidine. The diester thus has the optimal geometry required for ester links to nucleosides at each end, as required in NAD.

sides at both ends of the pyrophosphate. The chemical structures of these molecules along with experimental torsional angles are shown in Fig. 1.

The structure of NAD is also shown. There are a number of common features between the two nucleotide

coenzymes CDP-choline and NAD. First, the diester pyrophosphate backbone of the dinucleotide is the same as that present in CDP-choline including a formal negative charge on a phosphate. One end of the bridge ester links to a nucleotide just as in NAD. The other end forms an

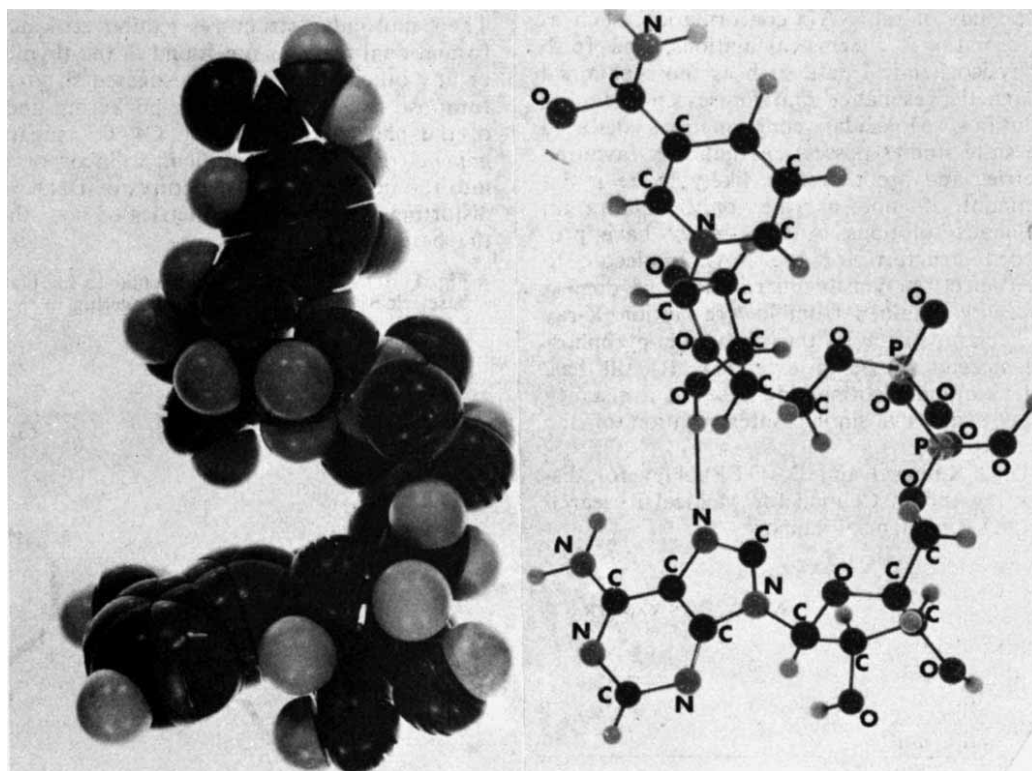


Fig. 2 Space filling and perspective views of the present NAD model.

ester bond with the choline moiety containing a quaternary nitrogen in CDP-choline whereas in NAD it is linked to the ribosyl nicotinamide moiety in which the nicotinamide is again present as a quaternary nitrogenous base. In CDP-choline the pyrophosphate is related to the nucleoside at one end and the choline moiety at the other by right- and left-handed (that is, opposite) rotations about the terminal ester bonds, respectively, as indicated by the sign of the torsion angles in Fig. 1a. Such a disposition of the pyrophosphate would also be expected if it were to link centrally two nucleosides instead of one nucleoside and one choline moiety. Examination of the various dihedral angles in CDP (Fig. 1) clearly demonstrates that its pyrophosphate geometry and orientation are simply those of CDP-choline, from the choline end the choline residue being replaced by the cytidine nucleoside. The pyrophosphate diester thus has the optimal geometry and conformation required for ester links to nucleosides at each end as in NAD and affords a reasonable basis for the proposed structure of the NAD molecule shown in Fig. 2.

Both the adenine and nicotinamide nucleosides of the model possess a *gauche-gauche* conformation about the C4'-C5' bond. This is consistent with the inherent stability of nucleotides with respect to this geometry⁸ as observed from X-ray data of nucleoside-5'-phosphates, dinucleosides and other nucleic acid fragments. The rigidity of this conformation is maintained in the presence of pyrophosphate and even triphosphate linkages as observed in the crystal structures of CDP⁷, CDP choline⁷, UDP (M.A.V., M. V. Hosur, M. L. Post and O. Kennard, unpublished), and ATP⁹. The adenine and nicotinamide residues in the proposed structure of NAD are too far apart for either internal stacking or hydrogen bonding. Note that in none of the structures of NAD analogues so far solved by X-ray methods^{5,6} or even in the cocrystallised salt of 1-methyl nicotinamide and aden-9-yl acetate¹⁰ is there any complementary or mutual hydrogen bonding between the nicotinamide and adenine moieties in spite of extensive hydrogen bonding found in the crystal structures.

The detailed nature of the model proposed here may facilitate further study of the NAD conformation, such as theoretical conformational energy calculations, and fresh assessment of physicochemical data such as those obtained from proton magnetic resonance and fluorescence studies of aqueous solutions. Molecular conformations deduced from crystalline state studies possess energetically favoured torsional geometries and are therefore likely to be maintained as dominant if not as the only equilibrium structures in aqueous solutions. Adams *et al.*² have proposed an extended structure for the NAD molecule to account for their electron density distributions of dogfish lactate dehydrogenase obtained from low resolution X-ray studies. In the present structure the diribotide pyrophosphate is folded instead of being extended. It still has, however, the adenine and nicotinamide bases at a distance of 10 Å and thus enables a similar interpretation of the electron density maps.

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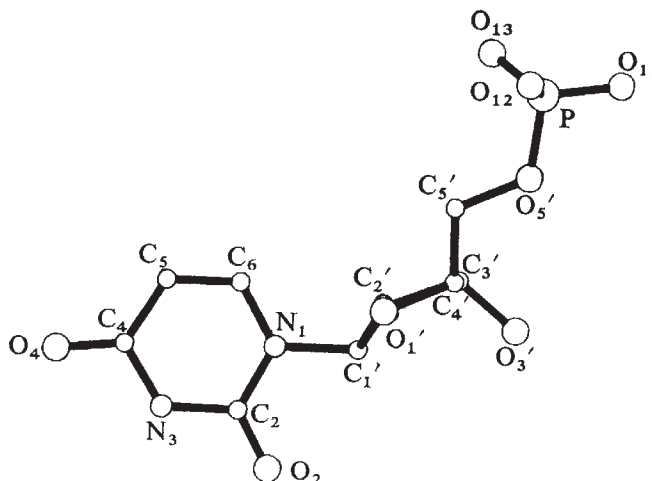
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An uncommon nucleotide conformation shown by molecular structure of deoxyuridine-5'-phosphate and nucleic acid stereochemistry

CRYSTAL structure determinations of nucleic acid fragments have shown that several of the conformational features found in the monomeric building blocks are also manifested at the nucleic acid level. Stereochemical variations between thymine and uracil nucleotides are therefore of interest as they can provide a structural basis for some of the differences between the conformations of DNA and RNA. X-ray studies have so far not shown any major dissimilarities between these two nucleotide species although the sugar ring of deoxyribonucleotides is found to possess greater flexibility than that in ribonucleotides. We report here the molecular structure of deoxyuridine-5'-phosphate (dUMP-5') which is not a common monomer unit of DNAs as it is replaced by its 5-methyl analogue deoxythymidine-5'-phosphate (dTMP-5'). The investigation was undertaken to help determine whether or not this implied a fundamental difference between the geometries of these two molecules.

The molecular structure of dTMP-5' has already been reported¹. Our study shows two crystallographically independent molecules of dUMP-5' in the crystal structure. Their molecular structures exhibit strikingly unusual conformational features not found in the thymine nucleotides^{1,2} or any other nucleotide investigated so far. The deoxyribofuranose ring shows C1'-*exo* puckering and the disposition of the phosphate about the C4'-C5' sugar bond is *trans-gauche* (*tg*). We find it difficult to incorporate these features into the double helical structures of DNA without seriously distorting the normal geometries of both the backbone and the base pairs.

Fig. 1 dUMP, viewed perpendicular to the plane of the uracil base, clearly showing C1'-*exo* puckering in the deoxyfuranose moiety.



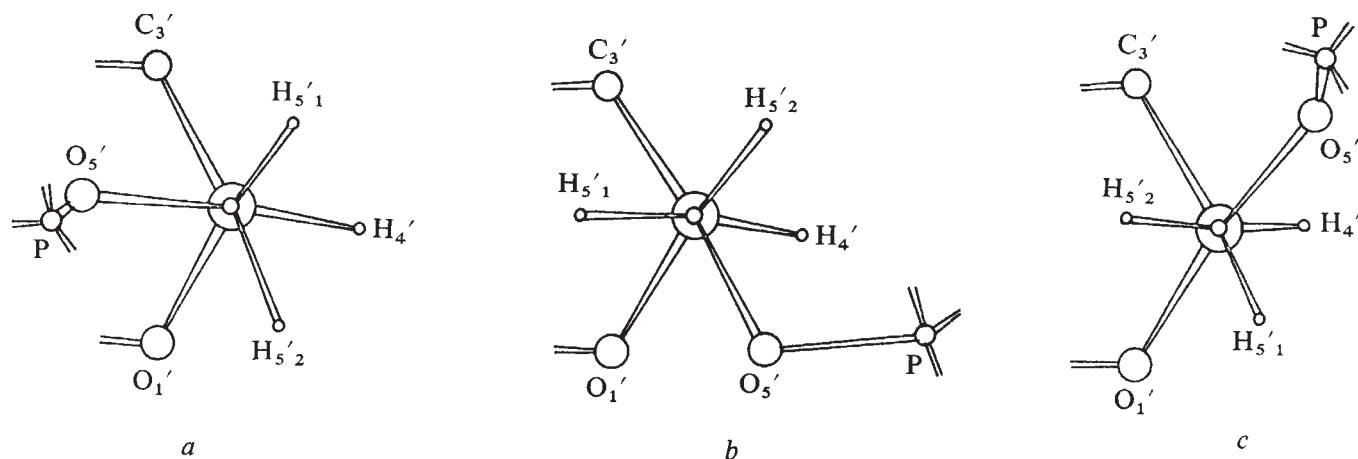


Fig. 2 The three possible staggered conformations of the phosphate moiety in nucleotides. Views, down the C5'-C4' bond, show the relevant region of: a, dTMP¹ (*gg*); b, dGMP^{9,10} (*gt*); c, dUMP (*tg*).

dUMP-5' was crystallised³ as a disodium salt from water-acetone solutions in the form of flat plates with monoclinic symmetry, space group $P2_1$ and cell constants $a=7.25$, $b=35.45$, $c=7.13$ Å, $\beta=102.2^\circ$. The measured density of $1.68 \text{ g cm}^{-3} \text{ ml}^{-1}$ was found consistent with the presence of two independent nucleotide molecules in the asymmetric unit with five waters of hydration per nucleotide. X-ray data were collected photographically using $\text{CuK}\alpha$ radiation and the intensities were estimated by visual comparison with a calibrated graded intensity scale. The location of the phosphorus vectors was not straightforward because of heavy overlap of peaks in the Harker section. The structure was solved by a judicious combination of Patterson superposition maps, direct methods and difference Fourier synthesis. It was refined by full matrix least square methods to a value of 8.9% for the conventional R factor. Where possible, the hydrogen atoms of the nucleotide molecules were fixed using their expected geometry.

The asymmetric part of the unit cell contains two crystallographically non-equivalent molecules of the nucleotide. The bond lengths and angles within them are consistent and comparable to those in other uracil nucleotides^{4,5}. Full details of the molecular dimensions and structure solution will be published elsewhere. The two

independent molecules show remarkable conformational consistency, the value of the dihedral angle $\text{O1}'\text{-C1}'\text{-N1-C6}$ being equal to 57.1 and 59.9° for the two molecules. The orientation of the uracil base with respect to the sugar ring is therefore *anti*, the only conformation about the glycosidic bond to be observed so far in crystal structures of nucleotides (for notations see ref. 6).

The best four atom least squares plane for the deoxyribose ring in molecule B passes through $\text{O1}'$, $\text{C4}'$, $\text{C3}'$ and $\text{C2}'$ atoms. The $\text{C1}'$ atom shows maximum deviation from this plane of 0.502 Å, on the side opposite the $\text{C5}'$ atom, compared with 0.017 Å shown by the $\text{C2}'$ atom. The sugar ring therefore has the $\text{C1}'\text{-exo}$ envelope conformation, clearly seen in Fig. 1. The sugar conformation in molecule A can be described $\text{C2}'\text{-endo-C1}'\text{-exo}$ as seen from the torsional angles given in Table 1. Those about the exocyclic $\text{C4}'\text{-C5}'$ sugar bond for the two molecules are: $\text{O5}'\text{-C5}'\text{-C4}'\text{-C3}' = -64.7$ and -65.9° , $\text{O5}'\text{-C5}'\text{-C4}'\text{-O1}' = 171.1$ and 172.2° . The conformation of deoxyuridylic acid in both the independent molecules is therefore *tg* about the $\text{C4}'\text{-C5}'$ bond. Figure 2 illustrates this geometry along with the other two staggered orientations about this bond, *gauche-gauche* (*gg*) and *gauche-trans* (*gt*).

Thus in having the *anti* conformation about the glyco-

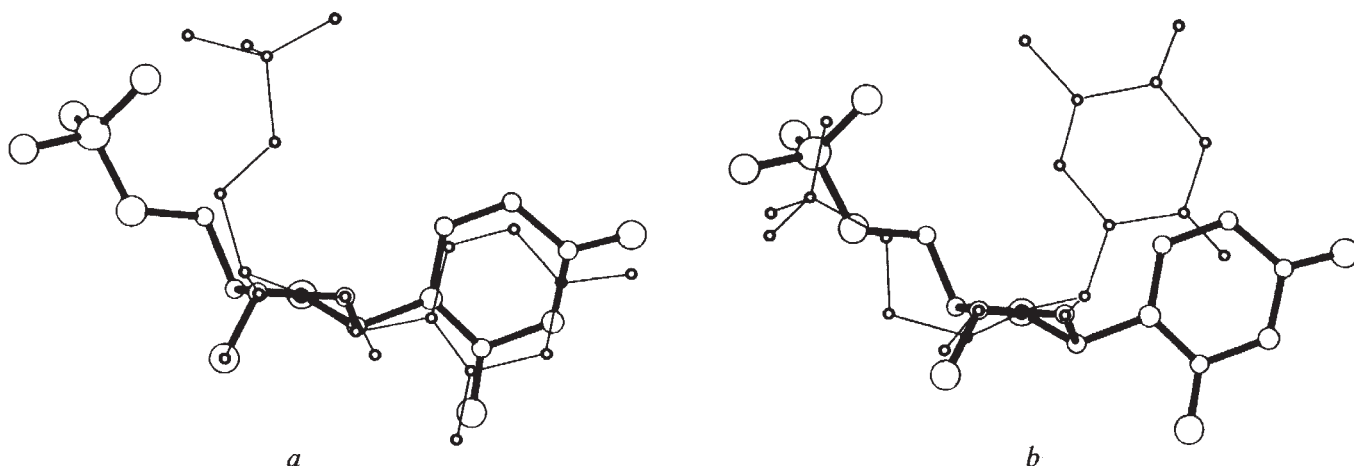


Fig. 3 Views of a, 5'-UMP (ref. 5) and b, dTMP¹ superimposed on dUMP (shown in heavy lines), to indicate the disturbance in overall molecular spatial character caused by *tg* and $\text{C1}'\text{-exo}$ conformation. In each case the view is taken down the bisector of the $\text{C2}'\text{-O1}'\text{-C3}'$ angle.

Table 1 Torsional angles

	Molecule A	Molecule B
C4'-O1'-C1'-C2'	-24.1°	-31.4°
O1'-C1'-C2'-C3'	30.8°	33.3°
C1'-C2'-C3'-C4'	-23.4°	-22.2°
C2'-C3'-C4'-O1'	10.3°	5.3°
C3'-C4'-O1'-C1'	8.9°	16.1°

sidic linkage, dUMP-5' is similar to dTMP-5', UMP-5' and all other nucleotides in the solid state. Its other two characteristics, the C1'-*exo* puckering of sugar and the *tg* conformation about the C4'-C5' bond, are fundamentally different from those of all the other nucleotides investigated so far. The most commonly observed conformation has been *gg* (Fig. 2a), whereas the *gt* conformer (Fig. 2b) has been observed in only two nucleotides, dGMP-5' (refs 9 and 10) and 6-azauridine-5'-phosphate¹¹ which is not a normal nucleic acid component. The refined models¹² of double helical DNA and RNA also possess the *gg* conformation about the C4'-C5' bond and C2'/C3' *endo* modes of puckering for the sugar. If the dUMP conformities were to be inserted into these structures, we suggest that this would considerably distort the normal stacking and hydrogen bond formation between the bases as well as the geometry of the backbone chain, thus exerting a destabilising influence on the double helix. The difficulty of incorporating *tg* (*g*⁻) conformation around the C4'-C5' bond into helical structures has also been realised in an earlier study⁸. It does seem, therefore, that the non-occurrence of this nucleotide in DNAs has a structural basis. The nature of changes brought about by the insertion of the dUMP conformation in the double helical structure may be seen in Fig. 3 which shows two perspective views of the dUMP structure superposed on the structure of UMP and dTMP.

There has been considerable interest in the study of the conformational freedom allowed in nucleic acids and their constituents. Based on careful surveys of crystal structure data, Sundaralingam⁷ has proposed that the nucleotide unit is essentially rigid, having a *gg* conformation about the C4'-C5' bond, and that the flexibility of polynucleotide chains is found mainly about the internucleotide P-O linkages. He has also envisaged alternative conformation for nucleotides generally correlated with less favoured sugar conformations⁸. Structural evidence on dGMP-5' (refs 9 and 10) and our results demonstrate the validity of this and establishes that significant flexibility about the exocyclic C4'-C5' sugar bond can exist, particularly when the sugar pucker is not restricted to the C2' and C3' atoms only. Although outside the preferred conformations found in nucleotides, these stereochemical features seem to be sufficiently relevant to nucleic acid structures, particularly when one has to consider the influence of local distortions in the double helix on the tertiary structure of DNA. Crick and Klug¹³ have made specific use of this property in their model for the highly folded structure of DNA in chromatin. They surmise that these foldings could be brought about by localised kinks in the double helical structure of DNA, where the backbone conformation is *gt* instead of *gg* about the C4'-C5' bond. Such a stereochemistry enables bending of the double helix while maintaining the integrity of the base pairs. In contrast, the *tg* conformation about the C4'-C5' bond together with the C1'-*exo* puckering of the sugar as found in the present structure, tend to disrupt the normal stacking and base-pairing geometry; and thus may be appropriate local conformations of DNA where separation and unwinding of the strands is presumably involved, such as during replication.

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Human endonuclease activity for DNA apurinic sites

APURINIC sites in cellular DNA may occur secondary to the slow hydrolysis of purines in physiological conditions¹ or the rapid hydrolysis of alkylated purines². Endonucleases which incise double-stranded DNA at apurinic sites have been purified from *E. coli*³, calf thymus⁴ and rat liver⁵. It has been proposed that such endonucleases hydrolyse the apurinic site as the first step of a repair process similar to excision-repair of ultraviolet photoproducts⁶. Lindahl and Nyberg calculated that spontaneous depurination, if unrepaired in postmitotic cells such as human neurones, would result in the loss of 3% of the total purines during a lifetime¹. Such a loss could be sufficient to produce abnormal proteins which appear in ageing cells⁷. The more rapid depurination of alkylated bases could be related to the mutagenic and carcinogenic properties of alkylating agents⁸. Therefore, to relate the repair of apurinic sites to the pathogenesis of human disease, we have characterised the properties of apurinic site endonuclease activity in crude extracts of HeLa cells and then measured such activity in WI-38 cells and human skin fibroblasts. We then compared enzyme activity in skin fibroblasts from individuals with two diseases in which repair of apurinic sites might be defective—progeria, characterised by premature ageing⁹ and associated with abnormal thermolability of cellular enzymes¹⁰, and Fanconi's anaemia, associated with an increased incidence of malignancy and *in vitro* susceptibility to chromosome breakage by alkylating agents¹¹.

We found apurinic site endonuclease activity in all human cell lines tested and no deficiency in progeria or Fanconi's anaemia cells. Apurinic sites were introduced into circular PM II phage DNA by heating at acid pH¹² and apurinic site endonuclease activity was assayed by measuring the conversion of super-helical DNA to the nicked form.

³H-thymidine-labelled PM II DNA was prepared and HeLa cells were grown as before¹³. WI-38 cells were obtained through the courtesy of Dr David Sabatini (New York University Medical Center). Both lines were determined to be free of *Mycoplasma* by uridine phosphorylase assay¹⁴. *Mycoplasma*-free normal skin fibroblasts (CRL-1295), progeria skin fibroblasts (CRL-1277) and

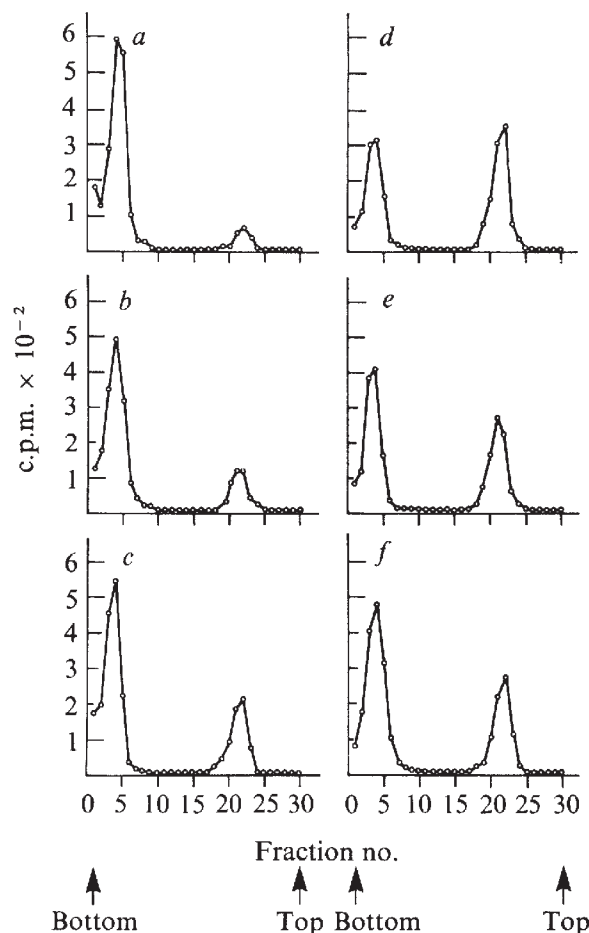


Fig. 1 Production of apurinic sites in PM II DNA. Aliquots of ^3H -thymidine-labelled PM II DNA in 0.1 M NaCl, 0.02 M Tris-HCl, pH 7.5, and 0.001 M EDTA were brought to pH 5.5 by addition of 0.1 M NaCl 0.1 M sodium citrate, pH 5.0 and 0.001 M EDTA, and heated at 70 °C in a water bath for the times indicated. The DNA solution was then neutralised to pH 7.2 by addition of 0.2 M Tris-HCl, pH 8.0 and 0.001 M EDTA. To ensure hydrolysis of all heat-induced apurinic sites, the DNA was then incubated at room temperature for 4 h in 1.0 M glycine-NaOH, pH 12.8 (ref. 12). This solution was then layered on top of a 4.0 ml CsCl gradient, density 1.50, pH 13.0, and 0.02% sodium dodecylsulphate that had been formed by centrifugation for 60 min at 20 °C at 37,000 r.p.m. in an SW 50.1 rotor in an ultracentrifuge (Beckman L2-65B). The gradients were then spun for a further 105 min at the same speed. Gradients were collected by the method of Carrier and Setlow²⁴ and counted in toluene POPOP. The direction of sedimentation is from right to left. At least 90% of the applied radioactivity was recovered and all of it was under the two peaks. The fraction of superhelical and nicked molecules was determined by summing the radioactivity under each peak. Breaks per PM II molecule were calculated from the Poisson distribution which yields the formula: No. of breaks = $-\ln x$ where x is the fraction of superhelical molecules²⁵. All apurinic sites were hydrolysed under these conditions¹² so that each break represents one hydrolysed apurinic site. *a*, control; *b*, 2.5 min; *c*, 5 min; *d*, 15 min; *e*, 10 min; *f*, 7.5 min.

Fanconi's anaemia skin fibroblasts (CRL-1196) were obtained from the American Type Culture Collection. All fibroblasts and WI-38 were grown as previously described¹³, with the exception of progeria fibroblasts which were supplemented with 20% foetal calf serum. WI-38 cells grew to $3\text{--}3.5 \times 10^6$ cells per 90-mm plastic dish, Fanconi's anaemia and normal skin fibroblasts to 2×10^6 cells per dish and progeria fibroblasts reached confluence at 10^6 cells per dish. WI-38 cells were received and assayed in passage 20–25. Fibroblasts were received frozen in early passage and assayed within 5–10 subsequent doublings. Cells were

prepared for assay by the method of Brent¹⁵ at 10×10^6 – 20×10^6 cells per ml. Protein was estimated by the method of Lowry *et al.*¹⁶.

Figure 1 shows the production of apurinic sites in PM II DNA. Each minute of heating produced 0.04 apurinic sites per molecule in the conditions specified. Figure 2a shows that the introduction of 0.6 apurinic sites per molecule did not produce breaks in the DNA. Figure 2b shows that the

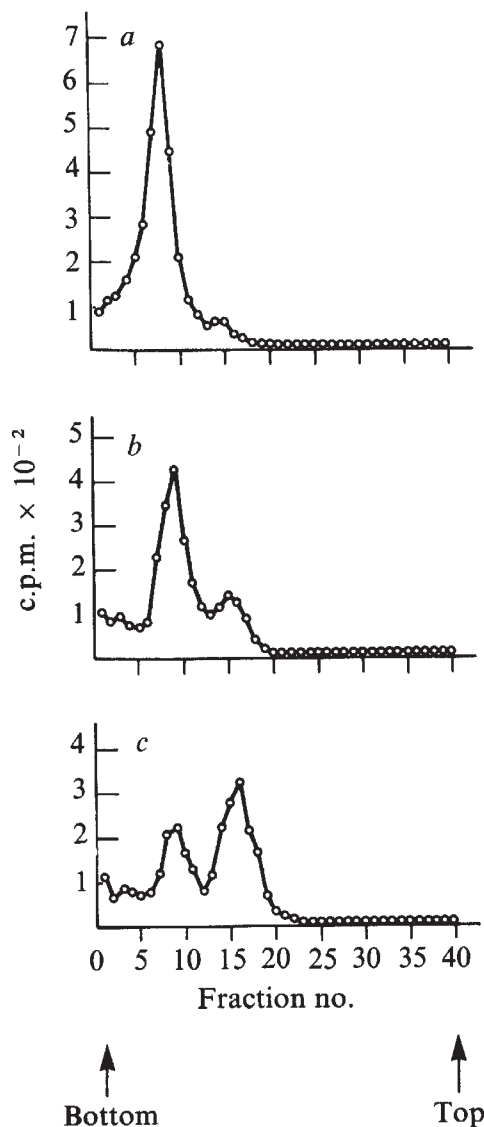


Fig. 2 Demonstration of apurinic site endonuclease activity in HeLa cell extracts. Aliquots of heated and subsequently neutralised DNA (0.3 µg) were incubated with HeLa extract of 100 µg protein (4×10^6 cells) for 30 min at 37 °C in a final volume of 120 µl. Conditions were identical to those described for ultraviolet endonuclease assay¹³. The reaction was stopped by chilling in ice and the entire mixture was then layered on to a 3.0 ml CsCl solution, density 1.50, pH 7.5, overlaid with light mineral oil and centrifuged in an SW 50.1 rotor at 43,000 r.p.m. for 2 h 45 min at 20 °C in an ultracentrifuge (Beckman L2-65B). Gradients were collected by the method of Carrier and Setlow²⁴ and counted in toluene POPOP. The direction of sedimentation is from right to left. More than 90% of the applied radioactivity was recovered and all of it was under the two peaks. Calculation of breaks per molecule as in legend for Fig. 1. *a*, DNA containing 0.6 apurinic sites per molecule without HeLa extract. *b*, DNA containing 0.2 apurinic sites per molecule with HeLa extract. *c*, DNA containing 0.6 apurinic sites per molecule with HeLa extract.

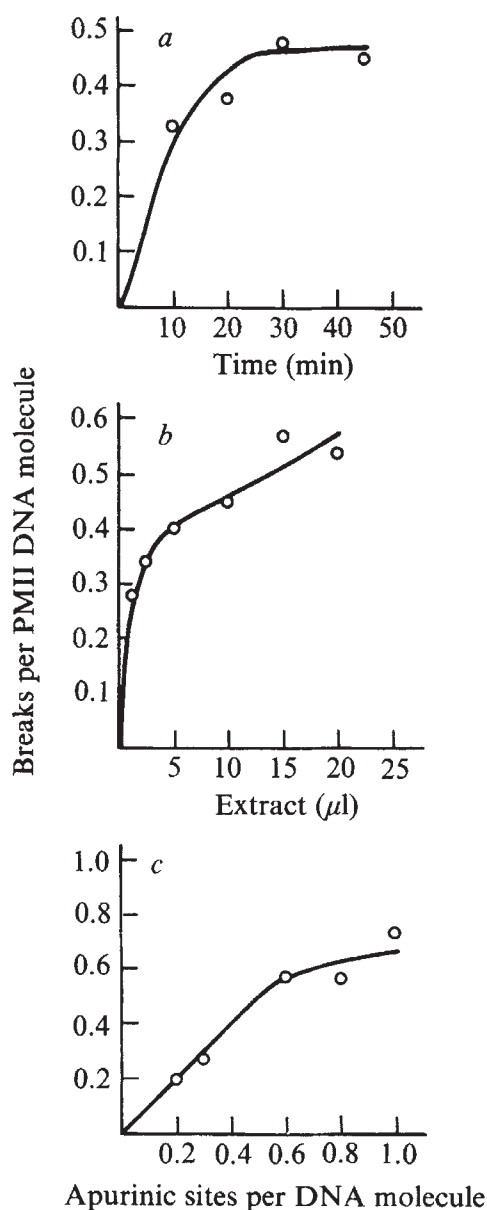


Fig. 3 Properties of HeLa apurinic site endonuclease activity. *a*, DNA (0.3 μ g) containing 0.6 apurinic sites per molecule was incubated with 100 μ g of crude extract. At the times indicated, the reaction was stopped by chilling in ice and the mixture was analysed by centrifugation as in Fig. 2. *b*, DNA containing 0.6 apurinic sites per molecule was incubated with increasing amounts of HeLa extract for 30 min and analysed by centrifugation. Protein content was 4.6 mg ml⁻¹. *c*, Relation between the number of breaks produced by the enzyme activity and the number of apurinic sites on the DNA. Samples of DNA containing the number of apurinic sites indicated on the abscissa were incubated with 100 μ g protein HeLa extract for 30 min and then analysed by centrifugation.

HeLa extract incubated with DNA containing 0.2 apurinic sites per molecule produced 0.2 breaks per molecule. Figure 2c shows that the HeLa extract produced 0.6 breaks per molecule when the DNA contained 0.6 apurinic sites per molecule.

Figure 3 summarises the properties of the endonuclease activity in crude extracts of HeLa cells. Figure 3a shows that the reaction reached completion within 30 min. Figure 3b shows that activity increased with increasing protein content. An extract of 3×10^5 HeLa cells produced breaks

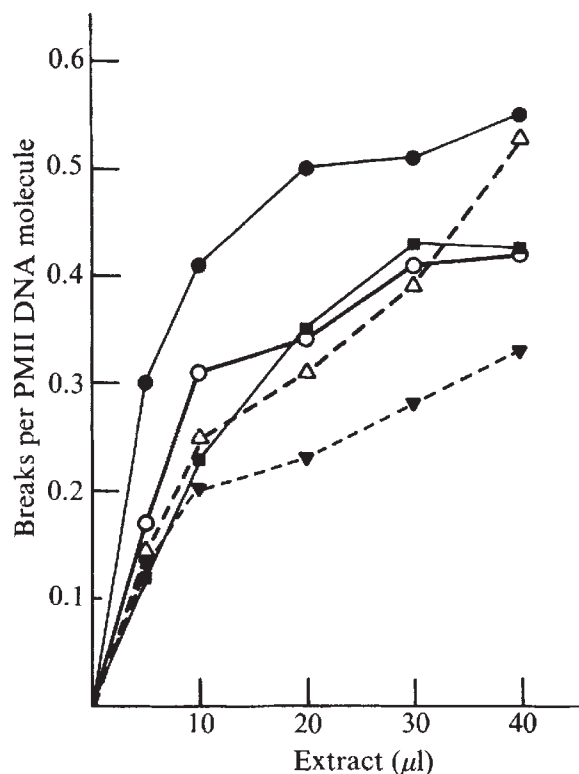
at every apurinic site. Figure 3c shows that the activity in an extract of 3×10^5 cells produced breaks for every apurinic site in a 1:1 ratio up to 0.6 apurinic sites per molecule and then plateaus.

Figure 4 compares apurinic site endonuclease activity of extracts of different cell lines. There was no deficiency of activity in progeria or Fanconi's anaemia cells.

These results demonstrate endonuclease activity against apurinic sites in DNA in crude extracts of several human cell lines. Therefore, there are at least two putative human repair endonuclease activities: one directed against ultraviolet damage^{13,15} and the other against apurinic sites. That these are distinct is suggested by the fact that apurinic site endonuclease activity is related to the number of alkali labile sites in DNA (Fig. 3c) while ultraviolet endonuclease activity is not^{13,15}. Purification of enzymes from calf thymus has yielded an apurinic site endonuclease with virtually no activity against ultraviolet irradiated DNA¹⁷, and an ultraviolet endonuclease with no activity against apurinic sites¹⁸. In contrast to our finding that ultraviolet endonuclease activity was much higher in HeLa and WI-38 than in primary skin fibroblasts¹³, apurinic site endonuclease activities were more similar in the cell lines.

The role of this activity in human DNA repair is uncertain. Actual repair of apurinic sites *in vivo* cannot be measured since there is no method for specifically introducing such sites in cellular DNA⁴. But the widespread occurrence of apurinic site endonucleases suggests that such lesions are repaired by cells⁹. That apurinic site endo-

Fig. 4 Comparison of apurinic site endonuclease activities of cellular extracts. PM II DNA was heated to yield 0.6 apurinic sites per molecule. Cell extracts were prepared at a concentration of 10^7 cells per ml. After 30 min of incubation, the reaction mixture was analysed by neutral caesium chloride sedimentation velocity centrifugation. Results are calculated as previously. Unheated DNA was not broken by 40 μ l of any of the cell extracts. ●, HeLa cells; ■, WI-38; ▼, normal skin fibroblasts (CRL-1295); ○, Fanconi's anaemia fibroblasts (CRL-1196); △, progeria fibroblasts (CRL-1277). Each point represents the result of an individual gradient except for CRL-1295 which are the average of three gradients.



nuclease activity is normal in progeria and Fanconi's anaemia cells indicates that either repair is normal, and that unrepaired apurinic sites do not have a role in the pathogenesis of these diseases, or repair might be abnormal due to a deficiency of other enzymes of the excision repair system. This possibility is suggested by a diminished rate of rejoining of single-stranded DNA breaks induced by ionising radiation in progeria cells²⁰ and a defect in exonucleolytic degradation of segments of DNA containing ultraviolet-induced pyrimidine dimers in Fanconi's anaemia²¹. It must be noted that the defect in progeria may be artefactual²² and the defect in Fanconi's anaemia is evident only at high levels of irradiation²¹. Finally, there may still be a repair apurinic site endonuclease deficiency in progeria and/or Fanconi's anaemia, but as shown for ultraviolet sensitive mutants of *E. coli*, multiple endonucleases may be present in crude extracts and measurement of composite activity would not reveal deficiency of the actual repair enzyme²³. Purification of endonucleases from human tissue and substrate characterisation is necessary to explain the role of these enzymes in the repair of genetic damage.

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IgM the secretory immunoglobulin of reptiles and amphibians

THE major secretory immunoglobulin in mammals (IgA) has been studied extensively but there is little direct evidence about its phylogenetic origins (reviewed in ref. 1). The degree of sequence homology between the C terminal end of human α_1 and μ heavy (H) chains suggests that the α and μ chains diverged ≈ 175 Myr ago, or at the time birds and mammals evolved from reptiles². Several reports suggest the existence in birds of an Ig analogous to mammalian IgA^{3,4}. We have therefore looked for a

comparable Ig in reptiles and amphibia. We found that these lower vertebrates do have a primitive secretory Ig, but it is similar to serum IgM.

Most of this study was concerned with Ig in the garter snake (*Thamnophis ordinoides*). This species has three antigenically distinct serum Ig classes: a 20.5S macroglobulin (called IgM) and two ≈ 9 S gamma globulins, Ig-1 and Ig-2 (our work in preparation with D. Leong and L. Thomas). Antiserum to snake serum Ig (IgM, Ig-1 and Ig-2) was prepared in rabbits by injection of snake anti-HEA antibody eluted from an affinity column. This antiserum was made specific for Ig-1 by absorption with normal snake serum (details will be described later).

Rabbit antiserum to snake bile was produced as described in the legend of Fig. 1; antiserum obtained 3 weeks after inoculation reacted with one fast gamma and two alpha serum proteins. Radioimmuno-electrophoresis of serum from snakes injected with HEA revealed that the gamma precipitin line specifically bound HEA ¹³¹I and fused in reaction of identity with the known IgM line developed with rabbit anti-snake Ig. Anti-bile antiserum was made specific for IgM by absorption with pooled anodal fractions from a Pevikon block electrophoresis⁵ of normal snake serum. Even after two boosters of bile only trace amounts of antibody to other serum gamma globulins were seen. In contrast, rabbit antiserum to purified serum IgM contained abundant antibody cross reacting with common antigens on 9S-Ig (probably light (L) chain determinants) (our work in preparation with D. Leong and L. Thomas). IgM was detected in bile and succus entericus (SE) by gel diffusion, but neither anti-bile IgM nor anti-serum IgM detected any antigenic differences between serum and secretory IgM. Thus, using the available antisera, no evidence was obtained for the existence of an extra antigenic fragment on secretory IgM.

Sucrose density gradient analysis (Fig. 1) revealed distinct differences in molecular size between serum and secretory IgM. Serum IgM was detected in a sharp concentration peak ($S \approx 20.5$ S) whereas the calculated S values for SE and bile IgM were more heterogeneous (IgM SE ≈ 15 S and 20.5S, IgM bile ≈ 17 S).

The IgM concentration in bile and SE, as determined by Mancini radial immunodiffusion⁶, was 14–25% that of serum. (These figures are an overestimate because of differences in molecular size of serum and secretory IgM.) Although IgM was not secreted against a concentration gradient, the data indicate that the presence of this Ig in secretory fluids was the result of selective transport. That is, using gel diffusion, IgM was the only Ig detectable in bile and the predominant Ig in SE (which also contained small amounts of Ig-1 and Ig-2). The relative concentrations of IgM and Ig-1 in SE were determined by the Laurell antibody-Agarose technique using rabbit antisera monospecific for bile IgM or serum Ig-1. Assuming passive diffusion of IgM and Ig-1 from serum to SE, the relative amount of Ig-1 in SE (that is, relative to the serum concentration) should be greater than that of IgM because of the smaller molecular size of Ig-1 (9S) compared with IgM (21S). The concentration ratio of SE-serum for IgM was, however, 56 times greater than that of Ig-1. To test the possibility that the predominance of IgM in external secretions was due to selective degradation of 9S-Ig, serum was diluted 1 : 1 with bile, SE or phosphate-buffered saline and incubated at 24 °C for 48 h. Using Mancini radial immunodiffusion with rabbit anti-snake Ig incorporated into the agar, no change in the ring diameters of IgM (inner) or Ig-2 (outer) were detected even after 48 h incubation. Furthermore, twofold dilutions of serum kept at 4 °C were compared by immunoelectrophoresis with similar dilutions of serum incubated as above with secretory fluids to determine the dilution at which the Ig-2 precipitin line was undetectable. Both control and experimental dilutions had similar and distinct Ig-2 precipitin endpoints of 1 : 32. These findings suggest that the relative abundance of IgM in bile and SE was a function of selective transport across the mucosa and was not due to loss of 9S Ig from the secretory fluid by proteolysis.

Preliminary evidence indicates a similar secretory Ig system

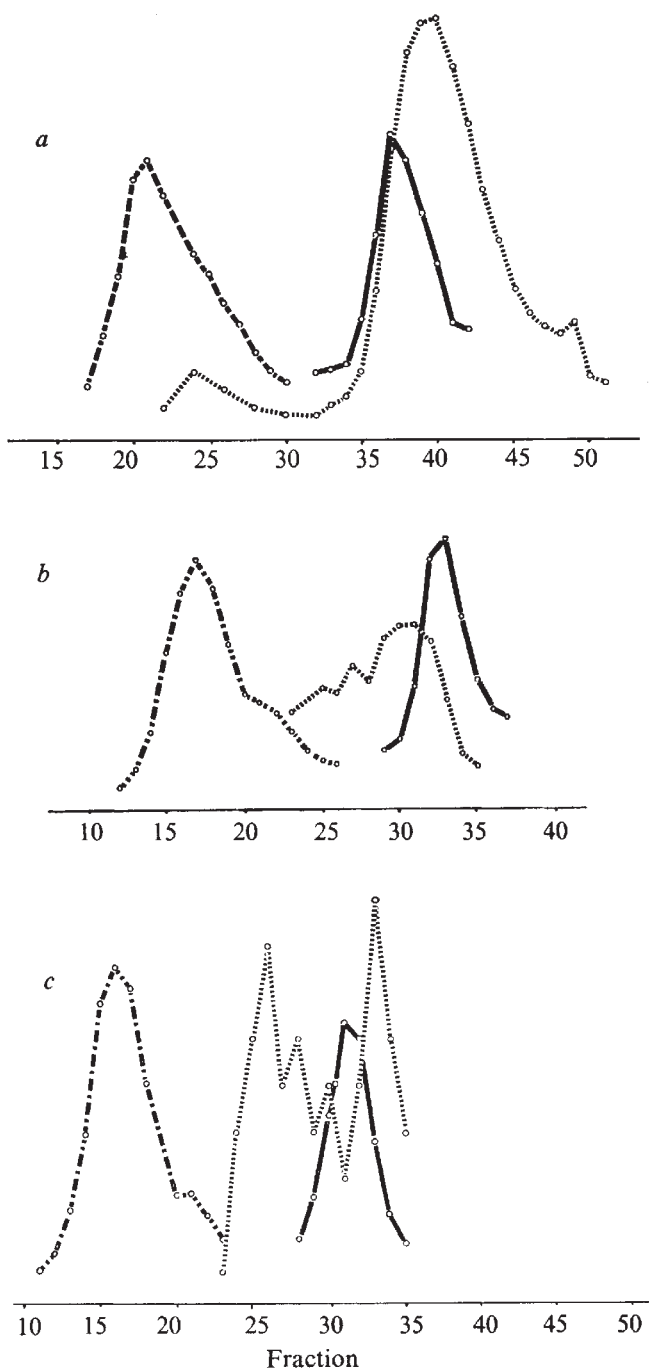


Fig. 1 Sucrose density gradient analysis of *a*, garter snake serum; *b*, bile; and *c*, succus entericus (SE). Bile was aspirated from the gall bladders of snakes and then pooled, dialysed and concentrated by negative pressure dialysis. SE was obtained from the intestines as described before⁶. 10–44% linear sucrose gradients were centrifuged for 18 h at 35,000 r.p.m. as described previously⁵ using ^{125}I -labelled rabbit gamma globulin (—•—) and ^{131}I -labelled hog thyroglobulin (—) as 7S and 19S markers, respectively. The fraction number is represented on the abscissa, relative IgM concentration and c.p.m. on the ordinate. Fractions (0.1 ml) were removed from the top of the gradients and analysed for IgM concentration by the Laurell antibody-Agarose technique⁸ using specific rabbit anti-garter snake IgM, produced by injection of 200 μl of concentrated bile (equivalent to neat bile from two snakes) in an emulsion prepared with equal volumes of M/15 PBS and Freund's complete adjuvant (Difco). Because emulsification of bile was very difficult it was necessary to prepare the emulsion in a Sorvall Omni-Mixer using 10 ml of PBS and 10 ml of adjuvant. Rabbits were injected subcutaneously at multiple sites and were boosted 3 and 6 weeks later with 100 μl of bile in incomplete Freund's adjuvant (Difco). After absorption (see text) the antiserum reacted only with IgM in serum or secretory fluids. The approximate S values of the IgM concentration peaks were: *a*, serum $\approx 20.5\text{S}$; *b*, bile $\approx 17\text{S}$; and *c*, SE $\approx 15\text{S}$ and 20.5S, IgM.

in turtles (*Chrysemys picta*) and bullfrogs (*Rana catesbeiana*) because two rabbits immunised with neat bullfrog bile and two rabbits immunised with turtle bile each produced antisera which reacted specifically with their respective serum IgM. None of the antisera detected antigenic differences between serum and secretory IgM. It was particularly interesting that rabbit anti-snake, bullfrog and turtle bile revealed no cross reaction between the serum IgM of these species. In contrast, bullfrog anti-rabbit gamma globulin showed broad cross reactivity with other mammalian gamma globulins⁶. This phenomenon is discussed further in ref. 6.

It is noteworthy that the production of antisera specific (except for anti- α globulins) for snake, turtle or bullfrog IgM was achieved simply by injection of rabbits with bile from the respective species. Purified snake serum IgM, however, elicited abundant cross reacting (L chain) antibody. We are investigating whether these specificity differences resulted from unique properties of secretory IgM or from differences between the immunising emulsions (see legend to Fig. 1).

Sequence analysis of human Ig has revealed marked structural similarities between IgM and IgA^{2,7}. These two Ig classes also resemble one another functionally, in that IgM can substitute for IgA in external secretions of patients with selective IgA deficiency⁸. The results of our study indicate that IgM served the function of a secretory Ig before the evolution of the α -heavy (H) chain gene. If sequence analysis of avian secretory Ig corroborates its homology with mammalian IgA, the lack of a unique class of secretory Ig in present day reptiles would place the evolution of the α -H chain gene in a high reptilian ancestor of birds and mammals.

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Corrigendum

The complete authorship of the article "Age of KBS Tuff in Koobi Fora Formation, East Rudolph, Kenya" (*Nature*, **258**, 395; 1975) should be:

G. H. Curtis, R. E. Drake, T. E. Cerling, B. L. Cerling and J. H. Hampel.

Erratum

In the article "Post-transcription control of isocitrate lyase induction in the eukaryotic alga *Chlorella fusca*" by A. H. Scragg, P. C. L. John and C. F. Thurston (*Nature*, **257**, 498; 1975) the second line of text below the legend to Fig. 2 should read . . . concentration was largely isocitrate lyase. This, in turn, demonstrated . . . and not as printed.

matters arising

Defensive stoning by baboons

COMMENTING that deliberate stone-throwing by baboons had not been previously recorded by professional field observers, Hamilton *et al.*¹ described the behaviour from a colony of Chacma baboons (*Papio ursinus*) in South West Africa. This is not, however, confined to *P. ursinus*; I saw essentially similar behaviour from Anubis baboons (*P. anubis*) when on a short visit to Beldong Forestry Nursery in the Jebel Marra Massif of the Sudan in December 1962. The nursery is in a sparsely populated area, and at the time of the visit, had been in existence several years. As far as I am aware, baboons were not hunted locally and, from their general behaviour, they seemed to be partially habituated to the presence of the local population.

The stone-throwing incidents occurred while I was photographing on a steep hillside at about 1,800 m, some distance from the nursery where the vegetation had been cleared from derelict cultivation terraces. While I had been working across the hillside a troop of baboons, seemingly attracted by my presence, approached cautiously, stopping at intervals, until they were on the second and third terraces directly above me. After a short time, during which the troop showed some agitation, the largest male started dropping stones down the slope towards me, and was later joined by two, sometimes three, of the older baboons. The stones were taken from the broken terrace edge and cast with an underhand shovelling motion, as described by Hamilton *et al.*¹. By repeated trials I found that more stones were rolled down when I was bent over the camera on its tripod than when I stood to one side of it, suggesting that the apparent threat came from the observer-camera-tripod complex. This received some support from the baboon behaviour when I finally tried to photograph them casting stones; when I took up the camera and tripod for a closer shot, the troop fled upslope with much barking, the majority going over the brow of the hill, though some of the largest animals lingered on the skyline when I did not follow.

I took no measurements of the rocks used by the baboons but my notes indicate a size similar to those used by *P. ursinus* (corrected data, see reply) and possibly somewhat larger, though in this case the selection would have been for smaller

ones from the generally large rocks of the terrace wall. The 'aim' was uncomfortably good much of the time, but fortunately the terrace immediately above me caught or deflected most of the stones bouncing down the slope.

There is a difference between this example of stone-throwing and that of *P. ursinus*. In the latter, stones were cast from the rocky walls of the canyon where the baboons slept and retreated to when threatened by predators. In the present example, the behaviour took place well away from the sleeping area and the observer-camera-tripod complex seemed not to be perceived as a threat until the baboons themselves approached. As stressed by Hamilton *et al.*¹, partial habituation to humans was probably an important factor in this behavioural response. Although I have seen other baboons in the Sudan and northern Nigeria on inselbergs where stone-throwing might have been possible, I have seen no other example since. Elsewhere in the Sudan, the baboons were probably well accustomed to the presence of humans. In northern Nigeria generally, baboons are hunted and extremely wary, though in Yankari Game Reserve where, in contrast, they are protected and tame, they do not respond to camera and tripod in this manner.

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¹ Hamilton, W. J., Buskirk, R. E., and Buskirk, W. H., *Nature*, 256, 488 (1975).

HAMILTON *et al.* wish to draw attention to the following errors which appeared in their letter (*Nature*, 256, 488; 1975): on page 489, line 9 should read 16.5 cm and 10.4 cm and not as printed; and in Table 1, last two lines should read SAM, subadult male and AM, adult male.

IN January 1973, I was conducting geological fieldwork in Baringo District. One afternoon I was approaching the fault scarp west of Lake Baringo, near Kampi-ya-Samaki, Kenya, with the intention of collecting some hyrax skulls which I had observed through binoculars when examining the nest of Verreaux's

Eagle (*Aquila verreauxi*). As I approached the cliff, a troop of about 15 baboons near the cliff started climbing it. My presence had obviously alarmed them, and a few individuals were barking with the typical "wahoo" call. I continued to approach the cliff and was almost at the top of the scree slope when a rock flew past my head and splintered just below me. At first I thought a baboon had dislodged a stone accidentally, but when I looked up to see where the baboons were, I saw another deliberately extend his right arm over the edge of the cliff and release a second stone. This happened several more times, but as I was scrambling down the scree to get away, I neglected to make a note of exactly how many stones were tossed towards me. It was, however, probably no more than ten.

The baboons had been about 50 m from me, parallel to the cliff when I first encountered them, but they deliberately scaled the cliff and then walked along the top so as to be above me when they released the stones.

My experience differs somewhat from that reported by Hamilton *et al.*¹, in that this was the first time I had ever seen this troop of baboons, so the troop was in no way habituated to me. The whole episode, from initial contact to my departure, was over in less than 5 min. In other respects, however, the behaviour was as described by Hamilton *et al.*

This record extends the stoning behaviour to Kenya, and as postulated by Hamilton *et al.*, the behaviour may be quite widespread. My native field assistant assured me that he had seen it several times, and said that baboons will attack goats and kill and eat them. I have never seen the latter, but have been told by many Tugen people that it is a common occurrence there.

I question whether the stoning behaviour should be labelled "defensive". There were definite connotations of "offence" in the behaviour I saw. In particular, I was over 50 m away from the baboons at first contact, and it was they who approached me along the cliff top. When the baboons first saw me they were evidently agitated, but once on top of the cliff were out of danger. Only a couple of baboons tossed stones, and to do this they had to walk over 50 m towards me. The tossing was accompanied by rapid eyebrow raising and a tense alert carriage on the part of the thrower. All other

baboons that I could see at the time were not unduly alarmed at my presence once they had reached the top of the cliff.

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Nature, 256, 488 (1975).

H-2 antigen restriction of viral infection

KOSZINOWSKI and Ertl demonstrated¹ that T lymphocytes immunised *in vivo* against virus kill virus-infected target cells only when these share H-2 antigens with the effector cells². The commonly expressed view³ is that the effector T cells recognise an antigen created by an interaction of H-2K or H-2D antigen molecules with viral protein.

To obtain further support for this notion, Koszinowski and Ertl show convincingly that anti-H-2 serum blocks killing when it is directed against the target cells, but not when directed against the effector cells. An unfortunate nomenclature (for example, anti-H-2^{kk} for (H-2^{d/k} × H-2^{bb}) anti-H-2^{kk}) gives the impression that the sera are directed against the antigens of the whole H-2 complex, when in fact both antisera used are directed only against the K end of the H-2 complex. Thus the H-2D antigens of the effector cells are still free, and one cannot rule out the alternative hypothesis⁴, that in order to kill, effector cells have to match up their SD antigens with those of the targets⁴.

On the other hand, cytotoxicity is blocked completely whenever the anti-serum, be it an anti-H-2K or an anti-vaccinia serum, is directed against the target cells, and this is taken as evidence for an interaction product being the target. It is important to know whether a similar blocking could be caused by any antiserum directed against the target cells, or only by anti-H-2 or anti-virus serum. Two points are of particular interest in this respect. First, blocking of cytotoxicity is complete although the antisera cover only the K end—if the blocking is as specific as in allograft reactions, where blocking the target antigens of only one end of H-2 hardly affects the killing⁵, this would mean that vaccinia, unlike LCM and ectromelia virus², modifies only the H-2K antigens. Second, one should note the blocking of F₁ target cells. For example, H-2^k effectors do not kill H-2^d targets, but they will kill (d × k)F₁ as well as H-2^k target cells. Killing of H-2^k is blocked by anti-H-2K^k, not by anti-H-2^d, while killing of F₁ targets are blocked by both

Matters arising

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antisera. If the H-2^k effectors interact only with modified H-2^k antigens on the target cells, one should, again by analogy to the specificity in allograft reactions⁶, not have expected anti-H-2^d serum to have any effect on the killing of F₁ target cells by H-2^k effector cells. My comments are based on the assumption that complete blocking is obtained only when all possible effector-target cell interactions are inhibited, and I have overinterpreted the data, if the relationship between percentage ⁵¹Cr-release and number of lytic interactions is less quantitative.

The field of T-cell mediated immunity to virus infected target cells is fruitful and holds promise of information about the evolutionary significance of histocompatibility antigens³. It is therefore important, before we make conclusions about mechanisms, to know to what extent the phenomenon is analogous to allograft immunity.

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² Blanden, R., et al., *Nature*, 254, 269 (1974).

³ Lennox, E., *Nature*, 256, 7 (1975).

⁴ Zinkernagel, R. M., and Doherty, P. C., *Nature*, 251, 547 (1974).

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KOSZINOWSKI AND ERTL REPLY—We think that our data¹ do not allow the interpretation that serum mediated blocking of cell-mediated cytotoxicity (CMC) of virus infected syngeneic cells reflects an inhibition of all possible effector-target cell interactions. We cannot express this opinion since we find inhibitory activities also with antisera directed against vaccinia virus specific surface antigens².

The F₁ fibroblast targets were extremely sensitive for vaccinia infection which led

to high spontaneous and small specific lysis. We are unsure whether the H-2^d inhibition in these experiments had a significance, since unspecific blocking occurred to some extent (see Fig. 1 of ref. 1). Anyway, xenogenic anti-target cell serum absorbed for H-2 had no specific inhibitory effect.

There are indeed indications that, in the experimental conditions we used the K end is the essential target. When we tested an anti-H-2D^k serum ((H-2^b × H-2^d) anti-K^d D^k) we found minimal blocking activities.

These results can be explained by two possible mechanisms. Vaccinia virus alters mainly K-end antigens so that these modified antigens can form a target for the T cell. On the other hand it is possible that T cells predominantly attack K-end alterations. Comparable results concerning different inhibitory activity on T-cell mediated killing by anti-K-end or D-end sera have recently been described in a tumour situation³.

In the ectromelia system some mouse strains (B10.A (2R), B10.A (4R)) react more strongly at the K end⁴. Shearer et al.^{5,6} describe effector cell activity against TNP-modified cells absolutely restricted to the K end in two mouse strains while other strains recognise the TNP-modified D end as well. Restriction of CMC can therefore be dependent on the target cell^{5,6}, the modifying agent and the mouse strain used, so that at present no generalised answer about K- or D-end specificity is possible.

We think that the intimacy hypothesis⁷ of matching unaltered self antigens is unlikely. In addition to the arguments of Zinkernagel and Doherty⁸ we found that the target antigen in the vaccinia infection for the CL has a close physical relationship to SD antigens¹. Second, enzymatic reduction of H-2 antigens reduces CMC without affecting anti-vaccinia antibody mediated cytotoxicity (Ertl and Koszinowski, unpublished) and third, expression of the H-2 alloantigens is altered detectably by reduced serum absorbing capacity and inhibited allogeneic CMC^{9,10}. The latter observation especially, argues against the possibility of matching normal self antigens as a prerequisite for the recognition of new antigenic determinants.

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² Koszinowski, U., and Thomssen, R., *Eur. J. Immun.*, 5, 245 (1975).

³ Germain, R. N., Dorf, M. E., and Benacerraf, B., *J. exp. Med.*, 142, 1023 (1975).

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reviews

THE sixth volume in this definitive series devoted to Newton's mathematical papers covers the most important period in his scientific career, the climax of which was the publication of the *Principia* in 1687. Dr Whiteside has collected mathematically significant extracts from Newton's manuscripts bearing on his researches in the theory of moving bodies and of gravitation, which were his main concern at that time. These comprised the dynamical theory of the motion of bodies under central forces and his attempts on this basis to explain the observed motions of comets and the Moon.

In August 1684, following a challenge from Christopher Wren to produce an explanation of planetary motions, Edmund Halley went to Cambridge to discuss the problem with Newton. That momentous visit revived Newton's interest in the general dynamical theory of moving bodies which had lain dormant since his correspondence with Hooke nearly five years previously on the theory of falling bodies. In his final letter to Newton, Hooke raised the key question of the path of a planet acted on by the attraction of the Sun and, in particular, asked whether an inverse square law of force could make the planet deviate from its instantaneous uniform motion into an elliptical orbit with the Sun at a focus.

Three months after Halley had put the same question to him Newton sent to London the first version of a tract, in Latin, on the motion of bodies in an

Falling bodies

The Mathematical Papers of Isaac Newton. Volume VI, 1684–1691. Edited by D. T. Whiteside, with assistance of M. A. Hoskin and A. Prag. Pp. xxxiv + 614. (Cambridge University Press.) £25.00.

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Newton by Vanderbank, Possession Royal Family

orbit. This has been reproduced by Dr Whiteside in the present volume, together with an English translation. It begins with a proof that Kepler's law of areas in generalised form can be deduced for a law of central force. Newton also showed that an elliptical

path can be described under laws of force varying either as the distance or as the inverse square of the distance, the centres of force being, respectively, at the centre of the ellipse and at a focus. Among other results, he deduced for the latter case the third law of Kepler. He also went on to establish theorems concerning the motion of a projectile in a medium, resistance being directly proportional to speed.

On seeing this tract, Halley hastened again to see Newton in Cambridge. He found him already revising and enlarging the text into a full-scale treatise on motion. In the next two years, with Halley's encouragement, the text developed into, at first, two selected books, and ultimately the three that constituted the first edition of the *Principia*. The texts printed in the present volume illustrate the way in which the first book of the *Principia* evolved. Dr Whiteside has also included manuscripts concerned with particular problems such as that of the solid of least resistance to motion along its axis in a uniform fluid, the approximate determination of a parabolic cometary path, and the annual advance of the lunar apogee.

The volume concludes with some extracts from a scheme for revising the *Principia* in the early 1690s that Newton later abandoned.

The present volume maintains the high standards of annotation and printing set by previous volumes.

G. J. Whitrow

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THIS book is written by a professional. Dr Wong has plenty of experience in analysing experimental data and he is fully aware that the sophistication of the algebraic treatment must be matched by the quality of the data.

The steady-state derivations of rate equations, which form an introduction, are rigorous and there are helpful tips even for those who have been teaching the subject for some time. Some of the nomenclature is unnecessarily different from the usual and some algebraic shortcuts may make the book difficult for the inexperienced. The author's critical judgement of different steady-state treatments will be very valuable for most of the potential readers. Dalziel coefficients are used with advantage. It is a pity, however, that Dalziel's

Enzyme kinetics

Kinetics of Enzyme Mechanisms. By J. Tze-Fei Wong. Pp. xii + 294. (Academic: London and New York, August 1975.) £7.80; \$20.25.

treatment is not also used as an extended example for three substrate reactions. The warning about the caution necessary when playing ping-pong with Cleland (p.113) should be found in more treatments of enzyme kinetics.

Although it is good to see that the author has not completely neglected transient and relaxation kinetics, this area is not treated satisfactorily. Detail is given of the lag phase treatment developed by the reviewer 20

years ago. This has turned out to be of much less practical application than the subsequent analysis of the transient formation of enzyme bound products. The considerable amount of useful information obtained from this latter use of rapid reaction techniques deserves more space. Similarly the examples given for relaxation studies are those found in other text books. More recent and varied references would have been appreciated.

The above criticisms are obviously very personal ones and should not be taken too seriously. I would certainly recommend to any graduate student and research worker, who has some basic understanding of kinetics and calculus, to use this book for at least part of his journey towards a professional training in enzymology.

H. Gutfreund

Dangling infinitives

Writing Scientific Papers in English: An ELSE-Ciba Foundation Guide for Authors. By M. O'Connor and F. P. Woodford. Pp. 108. (Elsevier Scientific: Amsterdam, 1975.)

A PHYSICS student at my University attends about 1,500 hours of lectures, tutorials and laboratory classes on physics and mathematics during his undergraduate career. On completing his research a postgraduate must write a 30,000 word thesis, and may publish several articles in scientific journals. In spite of this, there is no formal tuition at all on how to write papers and submit them for publication. As far as I know the situation is similar in other universities and in other scientific disciplines. It is therefore not surprising that the scientific 'literature' is frequently turgid, dull, ungrammatical and sometimes incomprehensible. A recent review article—written by experienced scientists—contained the following: "... these spectra will be discussed at length, shortly...", "... the fluorescence spectra does not...". The word "only" was consistently misplaced: "... such a symmetry is only provided by the interstitial site...".

These errors are relatively minor, and one knows what the authors are trying to say. But if the natives write like this, what hope is there for foreign scientists, who are compelled to use English to reach the international scientific community?

This is where *Writing Scientific Papers in English* comes in. Its title is a bit misleading, in that the book covers the whole process of publishing a research paper. The chapters are headed Planning, Preparing, Writing the First Draft, Revising, Refining, Typing, Submitting the Final Version, Responding to the Editor, and Correcting Proofs, the first Appendix summarises the book (Steps in Writing a Paper), Appendices Two to Four deal with Units and Abbreviations, and Appendix Five with Expressions to Avoid. Over the next two years a series of supplementary booklets will be published, dealing with the problems of particular language groups, and with the various disciplines of science.

The best piece of advice O'Connor and Woodford give is, "Begin at the end. Write down your conclusions as clearly, precisely and economically as you can, and relate them to the question or hypothesis you have been examining". Furthermore, "A scientific paper should not be the history of an enquiry, but its outcome". And "Never... submit for publication a paper that has already been published or accepted for publication elsewhere".

In our writing we are told to avoid dangling participles and infinitives, to use active rather than passive voice, and verbs rather than abstract nouns. We should write, "we believe" rather than "it is thought" when referring to our own ideas: this type of circumlocution has been called the "passive of modesty". And it is misleading, because readers do not know whether the opinion belongs only to the authors, or is generally held.

I thoroughly approve of this book. It is packed with good advice, and written in an enviably lucid and readable style. Every library should have a copy. And every scientist, from Professor to student, whatever his nationality, should read it. The best compliment I can pay the book is that I will follow all its recommendations when I write my next paper.

J. Walker

Ice book



Growth of ice from liquid phase

Ice Physics. By Peter V. Hobbs. Pp. xvii+837. (Clarendon: Oxford; Oxford University: London, February 1975.) £29.

IN recent years there has been a great advance in our understanding of the physical processes going on in ice. Although ice has been studied for many decades, there were many aspects of its behaviour that were not understood—in particular, why it has the structure that it does, what the high pressure phases of ice are, and why ice has the electrical and mechanical properties that it does. Not all of these problems have been completely solved, but the great advances made are worth bringing together so that workers in the field can assess the current state of knowledge and can see how work in the different areas may shed light on each other. In this book Hobbs has tried to do this, and the result is a very

large book which surveys the whole field, including virtually all the important work and summarising our present state of knowledge.

This book will therefore become an essential reference for anyone working seriously on the physics of ice. It has no competitor for this place. The only other book in this area, N. H. Fletcher, *The Chemical Physics of Ice* (Cambridge, 1970), is a much shorter book with a different purpose. Fletcher introduces ice physics as an example of chemical physics. He selects from ice physics certain areas of interest, and shows how we have learnt to understand them. It is a book suitable for a course, or as an introduction to some important areas of ice physics. Hobbs's book is much more inclusive. It would not be suitable for a course, although certain individual chapters might. It is in the tradition of the monograph summarising knowledge, and it seems to have achieved this to a remarkable degree.

The first of ten chapters deals with the solid phases of the water substance—bonding of water molecules, structure of ice Ih and hydrogen disorder, other phases of ice and amorphous ice and clathrate hydrates. The second chapter concerns electrical properties, including the theory of electrical point defects in ice developed to explain them. The third chapter considers optical properties including luminescence, infrared and Raman spectra and some of the practical consequences. The fourth chapter is devoted to mechanical properties—elastic, plastic and fracture—including the difference between single crystals and polycrystalline ice. The fifth chapter deals with thermal properties and diffusion, including the problem of the heat capacity and zero-point entropy of ice and the diffusion of vacancies in ice. A chapter on surface properties leads on to a chapter on nucleation of ice and two others on growth of ice from the vapour phase and from the liquid phase. The final chapter is on ice in the atmosphere.

The book is remarkably up to date, and even has an appendix which includes results of the International Symposium on the Physics and Chemistry of Ice (Ottawa, August 1972), the results of which were only published about a year before the present book.

The bibliography is arranged so that it can be used as an author index, in that after each reference (which is given in full with the title of each cited paper) the page numbers on which it is cited are given. The conventional subject index and an index to tabulated and graphic experimental data add to the usefulness of the book. It is strongly recommended to anyone who has reason to enquire what we now know about any of the physical properties of ice.

John W. Glen

Scientific basis for practical conservation

Conservation in Practice. Edited by A. Warren and F. B. Goldsmith. Pp. xix+512. (Wiley: London and New York.) Cloth £9.50; paper £4.50.

AFTER fifteen highly successful years, the University College, London, MSC course in conservation is still alive and well, and residing in Bloomsbury, a fact which is demonstrated by the publication of this collection of essays written by many of those who have been involved in the development and teaching of this course. The term 'conservation' has taken upon itself a considerable breadth of meaning in recent years and this is reflected in the wide subject coverage of the thirty chapters in this book. These range over the control of weather, coastlines and floods, the preservation of genes, habitat management (including a number of case histories, for example, heathlands, woodlands and moorlands), pesticides and derelict land reclamation. There are accounts of historical approaches to subjects such as soil erosion, and experimental approaches to problems such as human trampling. And, of course, there are the inevitable accounts of the politics and administration of conservation.

Books could be written about any one of these subjects; indeed books have been written about most of these subjects. It is inevitable, therefore, that many of these short (average 15 pages) essays should be superficial. There are occasions when simplification is taken to the point of inaccuracy, but this is fortunately rare. Perhaps the most successful articles are those which do not attempt to summarise their entire subject, but concentrate on well-documented examples from which general principles may be drawn. For this reason I found Hollis on river management, Norman Moore on pesticides and Vita-Finzi on soil erosion, particularly valuable. It is understandable that difficulties will arise when trying to write short articles on broad subjects aimed at "the layman as well as students, planners, and so on."

One might also question the use of the phrase *in Practice* in the title. Max Nicholson states proudly in the Foreword that the book "was written almost entirely by administrators, planners and official and university scientists without a park superintendent, a forester or a land manager among them". One wonders how this can begin to be a manual of practical conservation; in fairness, it is not. Rather it seeks to emphasise the necessity for a scientific basis for practical conservation.

On the political and administrative side, Nicholson's Foreword must rank as the most entertaining and frank contribution. He decries the recent splitting of the old Nature Conservancy and one cannot but sympathise with his views. One wonders, however, whether it might result in a greater research effort into the practical issues of conservation rather than theoretical ecology. Admittedly, it is difficult to separate the two—only Whitehall seems capable.

Overall, the book's greatest merit is that it draws into one volume contributions from such a variety of disciplines. Any student who picks up this book will find some fresh ideas and information from a field outside his immediate training and, in doing so, he will broaden his concept of conservation.

P. D. Moore

Bumper book on cognition

Exploration in Cognition. (A Series of Books in Psychology.) By Donald A. Norman and David E. Rumelhart. Pp. xvi+430. (Freeman: San Francisco, 1975.) \$13.50.

THIS is a sort of academic version of a *Bumper Holiday Fun Book*, recounting all the latest adventures of Professor Norman and his jolly chums by the sea in sunny San Diego. The chums are called the LNR Research Group and they are accompanied on their explorations by their pet computing system, MEMOD.

The book begins with a general discussion of the group's approach to the study of language and cognition, leading to a comparison of the mind with an "active structural network". The important characteristic of an active structural network is that it stores factual information—John is 6ft tall—in the same way that it stores knowledge about how to manipulate that information: how to find out whether John is taller than Bill. It is at the same time a table of facts, and a flowchart for a program that can use the facts in the table to make further deductions. In computer programming terms, it erases the distinction between programs and data.

The group uses the terminology of active structural networks to formulate theories about the strategies we use to carry out various mental tasks, such as recognising faces and playing board games. They also write computer programs to simulate networks, and compare the performance of these programs with that of human subjects. MEMOD, whose name stands for "memory model", is

specially designed to simulate active structural networks, and the book gives a clear discussion, at layman's level, of how it works.

In the last half of the book, various members of the group describe their own research projects. Palmer's discussion of ways in which knowledge of the world can guide visual perception, and Eisenstadt and Kareev's work on how people analyse board positions in the games of Go and Go Moku are especially interesting.

The main fault of the book as a whole is a tendency to let theoretical discussions trail off inconclusively with remarks to the effect that more work needs to be carried out in the area. This seems to be connected with an unwillingness to set up any criteria by which we might judge the adequacy of the group's whole approach, and in particular the use of active structural networks. They are not bothered if a particular theory formulated in terms of these networks doesn't work very well, because they can always formulate another one instead.

Perhaps they are right not to worry. It is clear that there is some interesting work being done in San Diego, and this book conveys the feeling that they are having a good time doing it.

S. D. Isard

Pictorial Key to Genera of Plant-Parasitic Nematodes

FOURTH EDITION, REVISED

W. F. Mai and H. H. Lyon

The only work of its kind, this fully illustrated, easy-to-use manual will be indispensable to students and others who wish to identify nematodes. The descriptive key, full-page photographs, generic descriptions, and characteristics of genera will aid users in learning to differentiate plant-parasitic nematodes from other types that also occur in soil and plant tissue, and to identify them as to genus. Also included are 1500 citations of publications on the taxonomy of the 66 genera and on the general taxonomy of nematodes, particularly plant-parasitic nematodes. 224 pages, £6.35 (paper).

CORNELL UNIVERSITY PRESS
2-4 Brook Street, London W1

Bumblebees . . .

Bumblebees. By D. V. Alford. Pp. xii + 352 + 48 plates. (Davis-Poynter: London, June 1975.) £25.

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Photo: Stephen Dalton/Natural History Photo Agency

BUMBLEBEES hold a particular fascination for the entomologist because of their social organisation and because they are important pollinators of the native flora and many of our crops. Consequently, their biology has been studied in depth and several monographs have been devoted to them, to which Dr Alford's comprehensive and up-to-date account is a welcome addition.

The layout of the book is good. There is a brief introduction outlining bumblebee anatomy and physiology followed by the three main parts of the book. Part I includes chapters on various biological aspects of colony life, including hibernation, colony initiation and development. The author has confined himself largely to nest biology and says relatively little of their foraging behaviour and ecological importance as pollinators. The author's particular interest is reflected in the large section devoted to the parasites and predators of bumblebees. Part II deals primarily with the British species; each is described with information on their classification, habits and distribution. In Part III methods of collecting individual bumblebees and of locating, transferring and housing their nests for observation are outlined. The appendix presents 26 distribution maps of the British species of bumblebees as prepared by the Biological Records Centre for the Bumblebee Distribution Maps Scheme, for which Dr Alford is collating data sent in by members of the Bee Research Association. A discussion of how the distribution of different species is changing would have been interesting but perhaps little is known. Lists of all the plant and animal species mentioned

in the text are given and there are comprehensive author, plant and animal indices and a bibliography of over 500 references to original papers.

The book is copiously illustrated with 26 colour plates, 63 black and white plates and over 200 line drawings. The photographs are, however, disappointing. In his determination to present photographs of all the British species, the author has included many of poor quality. The line drawings are clearly drawn and enhance the text, although many of them would appear more attractive if they had been reduced in size.

The volume is expensive. It will certainly be too highly priced for many individual pockets but should be a useful addition to specialist libraries.

Ingrid H. Williams

. . . and beetles

The Life of Beetles. Glyn Evans. Pp. 232. (Allen and Unwin: London, June 1975.) £6.90.

As one of my mentors used to say, "God made too many beetles". Until now there has been no book on the general biology of beetles in English, and such a book is therefore bound to fill a need. Dr Evans has not, however, written an impressive book. Much more information about them can be conveyed in 200 pages even without the use of "specialist jargon", the absence

of which sometimes does duty here for a lack of knowledge. The book lacks balance. For instance, more than a sixth of the text is a chapter on food and feeding habits. Here we find nothing about the vitamin, amino acid, and other nutritional requirements of beetles nor about the role of gut and intracellular symbionts or how beetles utilise such materials as cellulose and keratin. Some of the illustrations are good, but a few are exceptionally poor (for example, Fig. 3). The references are at the end of the book, but for some reason they are cited separately for each chapter. One consequence of this is that in a meagre bibliography no less than 15 titles occur two or even three times in seven pages. In a section "Further Reading" no less than nine titles from the bibliography are repeated, sometimes twice.

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In the introduction and elsewhere the role of the hard and rigid body-wall cuticle in the success of beetles is heavily stressed, but most beetles are larvae and most larval beetles have a soft and flexible body-wall. Taenidial thickenings are not lost in the finer branches of the tracheae (p. 51); they are present even to the ends of the tracheoles. The visible spectrum of insects does not end at blue-green (p. 43) but extends from far below 300 to about 750nm in a few. No other group of animals has so wide a spectrum. The larvae of the Dryopidae are said to be aquatic (p. 29), but there is no authentic instance of an aquatic larva in this family. In the Hydrophilidae silk is not produced by the Malpighian tubes (p. 68) but is secreted by colleterial glands. He suggests (p. 68) that the funnel of the cocoon of *Hydrophilus* is a "tail-piece" of no functional significance, but it was long ago shown that it enables the embryos and young larvae to use atmospheric oxygen while the cocoon is covered by water.

There is a very real need for a book on the biology of beetles that combines wide personal knowledge of the animals with extensive reading. The desire to write a book on beetles is not of itself enough.

H. E. Hinton

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Lucanus cervus (stag beetle), from a drawing made in 1805

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Snails, slugs . . .

Pulmonates. Volume 1: Functional Anatomy and Physiology. By Vera Fretter and J. Peake. Pp. xxix+417. (Academic: London and New York, April 1975.) £13.20; \$35.

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Mary Evans Picture Library

Snails meeting on a vine branch, from an etching by M. Giacomelli

FROM the title, I wondered momentarily whether Fretter and Peake have produced a pulmonate sequel to the superbly integrated and illustrated Ray Society volume, *British Prosobranch Molluscs* (Fretter and Graham, 1962). Time is perhaps not ripe for such a volume. Considering the present state of our knowledge of the pulmonates, a challenging and daunting task has been undertaken in producing the present work.

In her introduction Fretter compares some aspects of the functional anatomy of prosobranch, opisthobranch and pulmonate gastropods and briefly considers their possible relationships and the origin of pulmonates from prosobranch stocks. She draws attention to the frequent differences in terminology used by prosobranch and pulmonate workers.

This volume brings together eight reviews covering the fields of locomotion, respiration, alimentary canal, water relationships, nervous system, endocrinology, reproduction and development. These reviews have been contributed independently by different authors and thus differ in form, in the selection of their content and in the period of time over which the author ranges; and several concentrate on research of the last ten years, that is, subsequent to the writing of Wilbur and Yonge's *Physiology of Mollusca*. Often, more and clearer illustrations would have been welcome, but all authors provide extensive reference lists—surprisingly without titles.

A valuable feature of this book is the systematic index. Within the context of the book, it pinpoints those species for which substantial data now exists, whilst indirectly indicating the limited number of pulmonate species on which some physiological work has been carried out. Sadly we must await volume 11, which deals with "the ecology of the group, some aspects of its economic importance, systematics and evolution", to relate the species utilised and the results they yield to their ecological niche and their systematic position. For convenience, a summary of the classification of pulmonates should have been included in this volume.

The authors are frank when presenting conflicting results—for example, page 291 "... contradictions can be expected by considering that not only different species were used, but that also the experimental conditions were not uniform . . .". Comments on problems, and the vast amount of work still necessary to unravel the complexities of functional anatomy and physiology among the pulmonates certainly make this book a stimulant to further research. As a record of what has been achieved in many areas and a pointer to what can be done, this book should take its place on the shelves of research departments.

Joyce E. Rigby

. . . and worms

The Locomotion of Soft-Bodied Animals By E. R. Trueman. Pp. viii+200. (Edward Arnold: London, June 1975.) Boards £8.50; Paper £4.25.

LIFE became mobile when animals evolved. Their movement, however, brings to mind the flight of birds or insects, the speed of horses, the mastery of swimming by fish and whales. But such creatures with highly efficient muscles working in antagonism to rigid internal or external skeletons are final productions of evolution. Humbler animals must be contemplated if we are to appreciate how mobility evolved and is still practised by major sections of the animal kingdom searching for food or seeking protection.

This book deals with movement in coelenterates, in worms from turbellarians to polychaetes, with some concern for echinoderms and much for molluscs. In evolution of the Metazoa, the solid flattened body suitable for progression with cilia over hard surfaces gave place to a rounded form with fluid-filled cavities—coelom, haemocoel or pseudocoel—before soft substrates could be penetrated. An infauna exploring new

sources of food and avoiding the attention of evolving carnivores came to supplement the original epifauna.

A muscular system acting in antagonism to a fluid skeleton provided a capacity to burrow—most simply exhibited by anemones such as *Peachia*—and more elaborately in various worms, and to the highest degree in the segmented polychaetes. Protrusion of the muscular foot with extensive haemocoelic cavities has enabled molluscs, primarily bivalves but also scaphopods and various gastropods, most efficiently to progress through soft substrates.

The process of movement in surface-crawling worms and snails has long been studied but the interpretation of movement within the substrate had to await elaboration of suitable measuring techniques, notably the Statham pressure transducer. So equipped, Professor E. R. Trueman and co-workers have revealed the sequence of events in the subsurface movements of representatives of all types of soft-bodied burrowers. Certainly to this reviewer, their success seems to be greatest where knowledge was least, in the interpretation of the digging cycle in bivalves leading to boring into rock or timber. The supreme achievement of movement in soft-bodied animals is, however, the jet propulsion of the molluscan squids, involving an elaborate system of antagonistic muscles under precise nervous control.

This concise, well written and illustrated book associates and interprets a range of experimental data to provide functional interpretation of much invertebrate structure. No one can read it without gaining a better understanding of the animal kingdom, surely the prime aim of the study of zoology.

C. M. Yonge

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Schistocerca gregaria (desert locust). Taken from Borne on the Wind: the Extraordinary World of Insects in Flight. By Stephen Dalton. Pp. 154. Hogarth: London, October 1975.) £5.00.

Announcements

Award

The Belgian Academy of Medicine has awarded the Pfizer Prize 1972-1973 to **Professor Jacques E. Dumont** of the Institute of Interdisciplinary Research, School of Medicine, University of Brussels.

Appointments

Dr Arturo Gomez-Pompa has been elected chairman of the International Co-ordinating Council of UNESCO's Man and the Biosphere Programme.

Dr Frank Read has been made a Professor in the Department of Physics at the University of Manchester.

Professor Sela has been inaugurated as president of the Weizman Institute.

International meetings

January 28-29, **The northern great barrier reef**, London (The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG, UK).

February 2-6, **Nuclear techniques in animal production and health as related to the soil-plant system**, Vienna (H. D. Hamel, Joint FAO/IAEA Division of Atomic Energy in Food and Agriculture, Kärntner Ring 11, P.O. Box 590, A-1011 Vienna, Austria).

February 4, **Replacement of biological methods by chemical and physical methods**, London (Chemical Society, Burlington House, London W1V 0BN, UK).

February 5-7, **Applied clinical immunology**, Houston (The Office of the Director, The University of Texas Health Service Centre at Houston, Division of Continuing Education, P.O. Box 20367, Houston, Texas 77025, USA).

February 11, **Techniques for the determination of traces of volatile compounds**, London (Chemical Society, Burlington House, London W1V 0BN, UK).

February 18, **Analysis of pesticides**, Chesterford Park (Chemical Society, Burlington House, London W1V 0BN, UK).

February 18, **Adhesion of thin films**, London (The meetings officer, Institute of Physics, 47 Belgrave Square, London SW1X 8QX, UK).

February 18-19, **Contraceptives of the future**, London (The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG, UK).

February 26-27, **Methods and applications of ranging to artificial satellites and the moon**, London (The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG, UK).

Reports and publications

Mammals of the Wankie National Park, Rhodesia. By V. J. Wilson (Museum Memoir, No. 5). Pp. 147. (Salisbury: The Trustees of the National Museums and Monuments of Rhodesia, 1975.) [1010]

Congress Against the President, Edited by Harvey C. Mansfield, Sr. Pp. 200. (New York: The Academy of Political Science, 1975.) [1010]

Smithsonian Contributions to Zoology, No. 202: Cyclopoid Copepods (Nanaspidae and Sabelli-philidae) Associated with Holothurians in New Caledonia. By Arthur G. Humes. Pp. iii + 41. (Washington, DC: Smithsonian Institution Press, 1975. For sale by US Government Printing Office.) [1010]

Publications of the United States Naval Observatory, Second Series, Vol. XXIV, Part 1: Third Catalog of Trigonometric Parallaxes of Faint Stars. By R. S. Harrington, et al. Pp. 30. (Washington, DC: US Government Printing Office, 1975.) [1010]

Smithsonian Contributions to the Earth Sciences, No. 13, Distinctive Properties or Turbiditic and Hemipelagic Mud Layers in the Algeiro-Balearic Basin, Western Mediterranean Sea. By Nicolaas A. Rupke and Daniel Jean Stanley. Pp. iii + 40. (Washington, DC: Smithsonian Institution Press, 1974. For sale by US Government Printing Office.) 95 cents. [1310]

United States Department of the Interior: Geological Survey, Bulletin 1395-H, Geologic Setting of the Glacier Peak and Mazama Ash-Bed Markers in West-Central Montana. (Contributions to Stratigraphy.) By R. W. Lemke, M. R. Mudge, Ray E. Wilcox and H. A. Powers. Pp. iii + 31. Professional Paper 877: The Black Hills-Rapid City Flood of June 9-10, 1972: A Description of the Storm and Flood. By Francis K. Schwarz, Lawrence A. Hughes, E. Marshall Hansen, M. S. Petersen and Donovan B. Kelly. Pp. vi + 47. (Washington, DC: Government Printing Office, 1975.) [1310]

Smithsonian Contributions to Zoology, No. 206: The Echinoids of Carrie Bow Cay, Belize. By Porter M. Kier. Pp. iii + 45. (Washington, DC: Smithsonian Institution Press, 1975. For sale by US Government Printing Office.) [1710]

Theories of Nature and Physics. By Herbert A. Bosch. Pp. 40. (Box 12, Little Falls, Minnesota: Herbert A. Bosch, 1975.) [1710]

United States Department of the Interior: Geological Survey, Water-Supply Paper 2123: Surface Water Supply of the United States, 1966-70, Part 8: Western Gulf of Mexico Basins, Vol. 2: Basins from Lavaca River to Rio Grande. Pp. ix + 861. \$5.70. Water-Supply Paper 2134: Surface Water Supply of the United States, 1966-70, Part 13: Snake River Basin. Pp. ix + 821. \$5.50. Water-Supply Paper 2155: Quality of Surface Waters of the United States, 1970, Part 6: Missouri River Basin. Pp. xi + 554. (Washington, DC: Government Printing Office, 1974 and 1975.) [1710]

Answers to Christmas quiz

1. (a) Not necessarily (see *Nature*, 256, 206; 1975).

(b) Nägeli the botanist and other naturalists failed to recognise their importance; Mendel became involved with administration on his appointment as abbot of his monastery, and as his girth expanded Mendel found it increasingly difficult to reach his pea plants (especially the short breed).

2. Norbert Wiener entered college at the age of 11 and gained his PhD at 18. The others were retarded to the extent of only starting college at 14/15 and not obtaining their doctorates until 21/22.

3. Both had to overcome a severe physical handicap. Sumner had his left arm amputated as a teenager. Cornforth has been completely deaf from birth.

4. The female deep sea angler fish can be 500,000 times heavier than the male.

5. Notorious, directed by Alfred Hitchcock. Professor R. A. Millikan told him that the whole idea of an atomic bomb was ridiculous, but the FBI still kept Hitchcock under surveillance for 3 months.

6. (a) *Twenty Thousand Leagues Under the Sea* by Jules Verne;

(b) *The War in the Air* by H. G. Wells;

(c) *The Unparalleled Invasion* by Jack London;

(d) *The Space Merchants* by Frederick Pohl and Cyril Kornbluth.

7. Letters and postcards between Albert Einstein and Willem de Sitter were found at Princeton and Leiden. They discussed the nature of the Universe.

8. In January this year Dr J. D. Isaacs and colleagues suggested that cars driven constantly on one side of the road can create atmospheric vorticity, leading to the formation of tornadoes (see *Nature*, 253, 254; 1975).

9. When deprived of vitamin B₁₂, some bats develop symptoms of demyelination. They may provide a useful system for research (see *Nature*, 254, 148; 1975).

10. Amphetamine, synthesised in 1927, was first used clinically in 1935. It produces a state of well being, decreases appetite and increases physical activity.

11. There is an excess of persons born in the first 3 or 4 months of the year in samples of schizophrenic

patients. This is called the season-of-birth effect.

12. In 1665 *Philosophical Transactions* was founded by Henry Oldenburg, Secretary of the Royal Society.

13. David Davies, editor of *Nature*, seen through the eyes of daughter Rebecca (see *Nature*, 254, April 3; 1975, front cover).

14. Charles Wheatstone (see *Nature*, 256, 532; 1975).

15. Members of the National Astronomy and Ionosphere Center at Cornell University in a message beamed to globular cluster M13, 24,000 light years away. *Nature*, failing to appreciate the subtleties optimistically aimed at our potential cosmic correspondents, printed the pictorial message the wrong way up. (see *Nature*, 253, 230; 1975).

16. Most people working on the structure of chromatin (see *Nature*, 257, 177; 1975).

17. (a) Miguel de Unamuno, *The Tragic Sense of Life*, page 90;

(b) O. W. Holmes, *Medical Essays*, page 211;

(c) Plato, *Theoetetus*, section 1.82;

(d) Adam Smith, *The Wealth of Nations*, book 5, part 3, section 3.